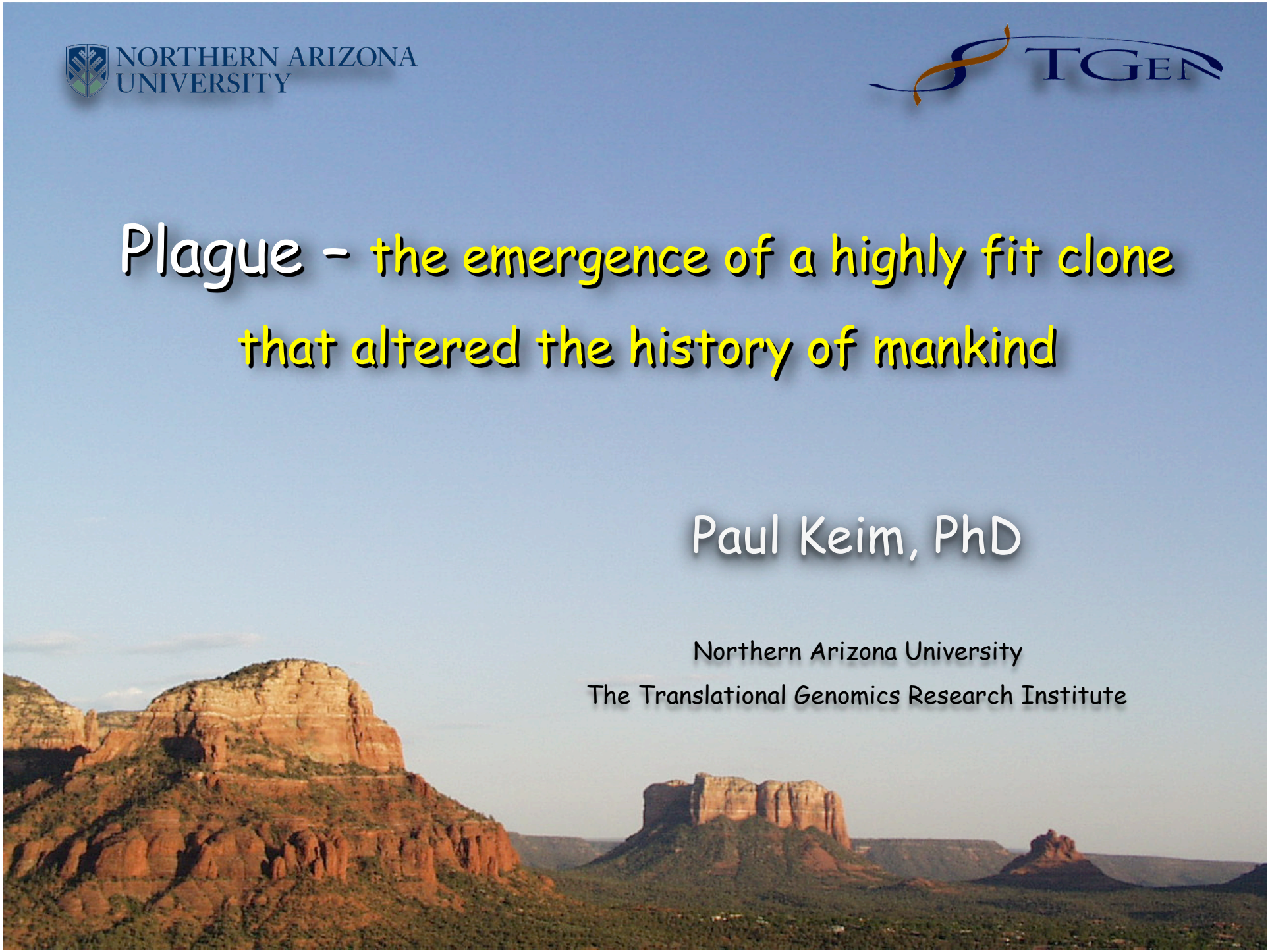

NORTHERN ARIZONA UNIVERSITY
TGEN
Plague – the emergence of a highly fit clone that altered the history of mankind
Paul Keim, PhD
Northern Arizona University
The Translational Genomics Research Institute

Welcome to New Mexico - Plague Central

SUSANA MARTINEZ, GOVERNOR | CARING FOR NEW MEXICO | CATHERINE D. TORRES, M.D., CABINET SECRETARY

For immediate release:
May 31, 2012

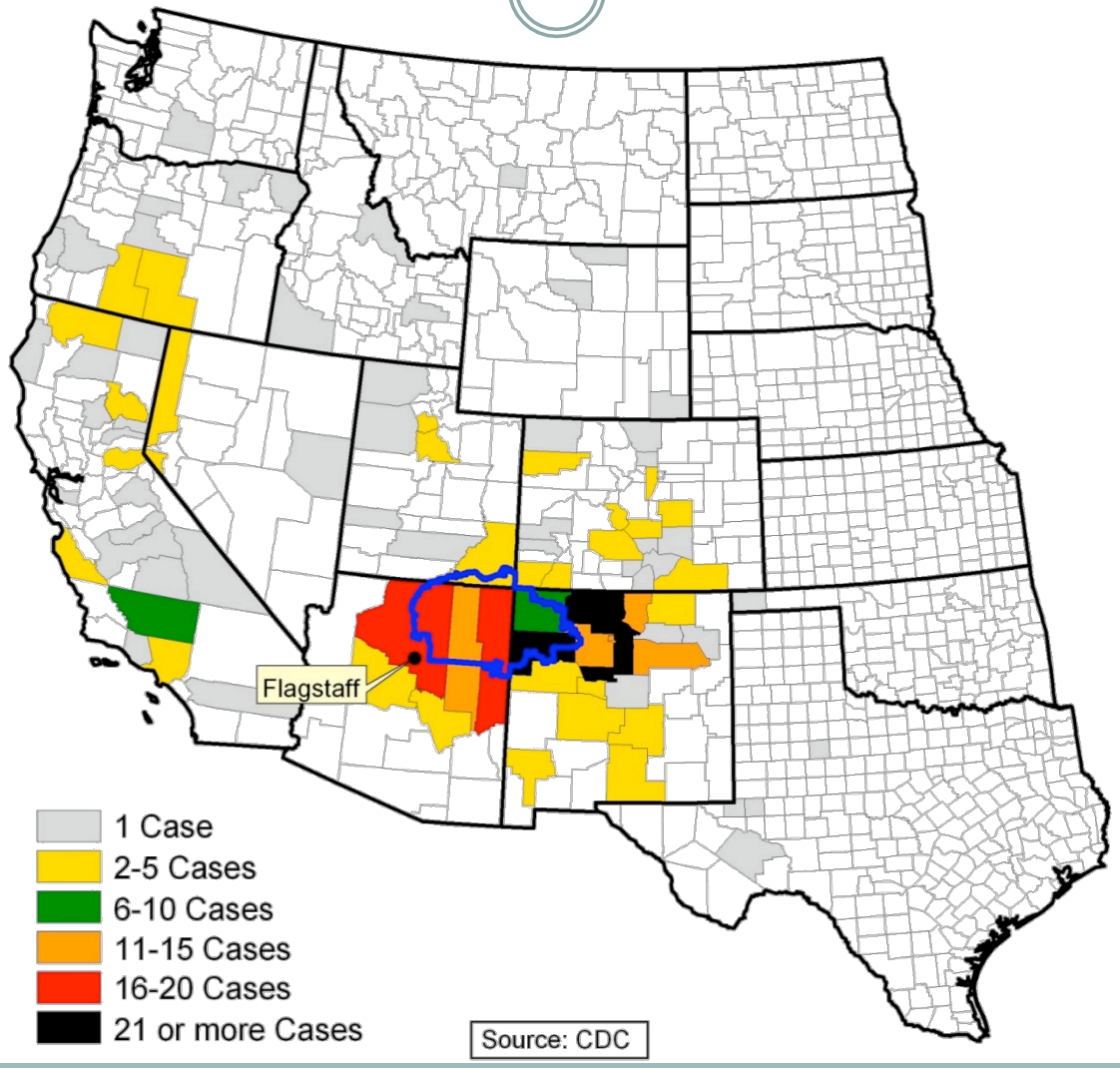
Media contact: Aimee Barabe
Cell: 505-470-2290

Department of Health Reports Plague Case in Torrance County Man

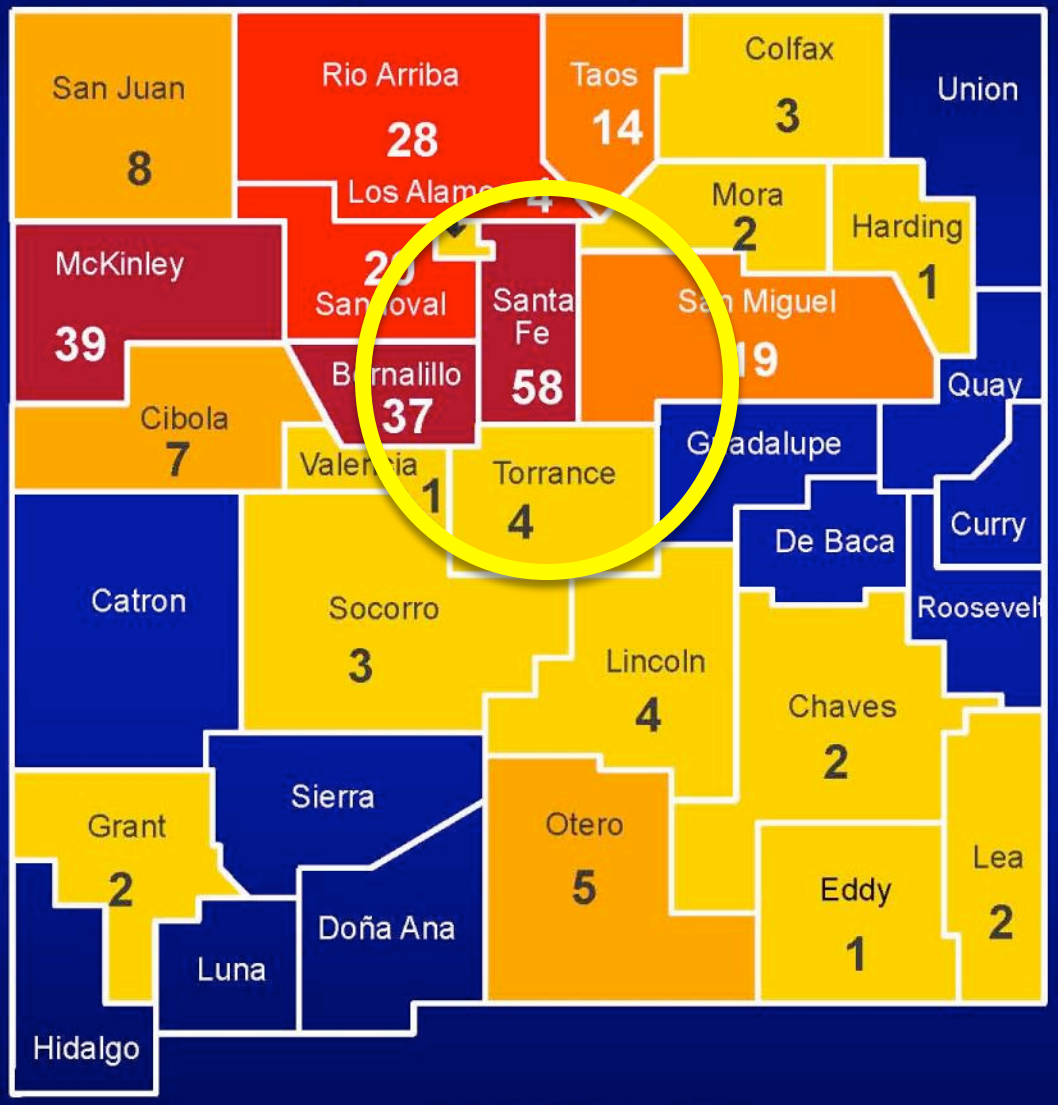
First Case in New Mexico and the United States in 2012

(Santa Fe) -- The New Mexico Department of Health confirmed today a case of plague in a 78-year-old man from Torrance County who is currently hospitalized in stable condition. This is the first human case of plague in New Mexico this year and in the United States. An environmental investigation will take place at the man's home to look for ongoing risk to others in the surrounding area.

Human Plague in the US: 1970-2000



Human Plague in New Mexico
Cases by County 1949–2011

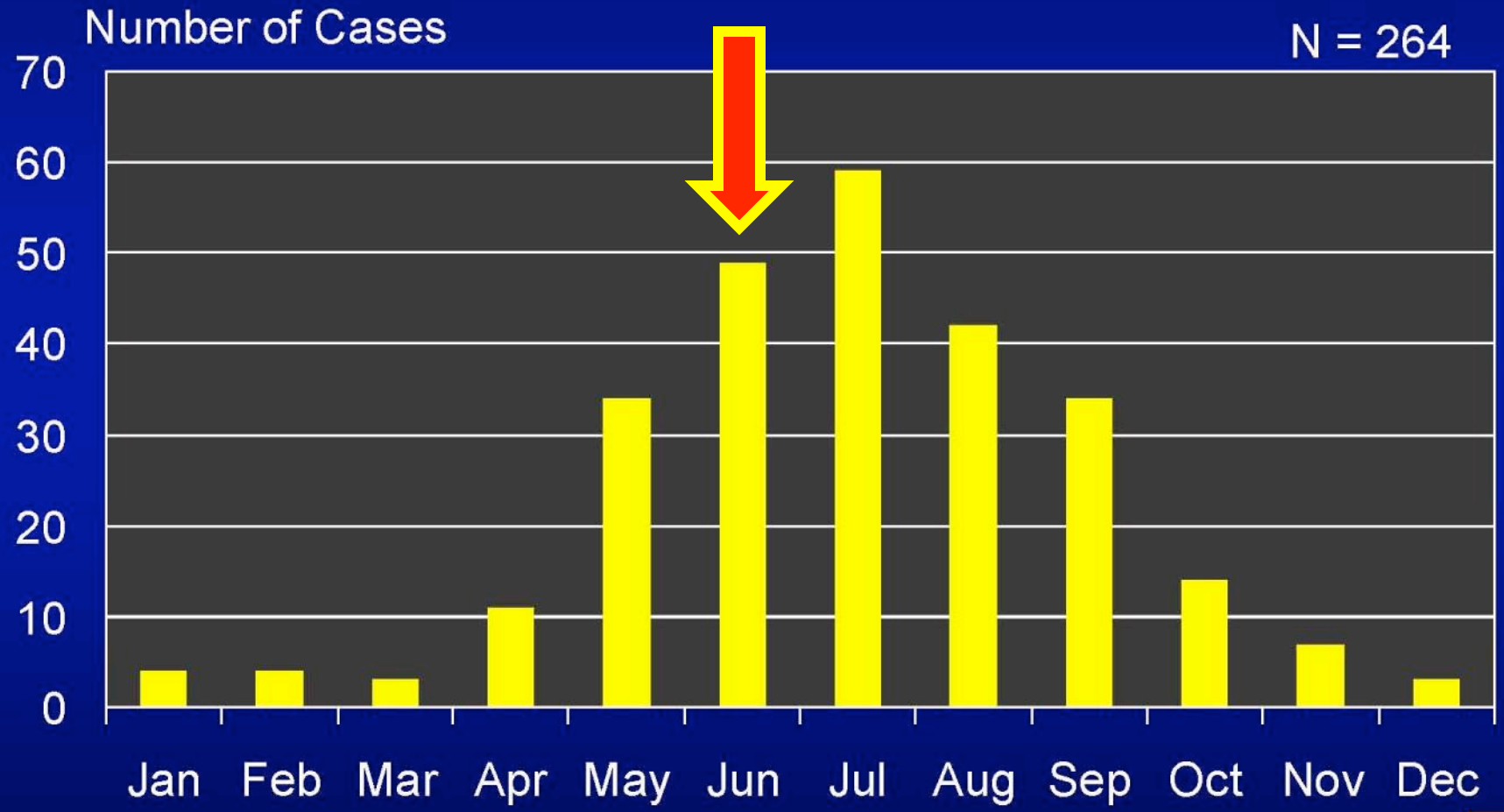


Number of Cases

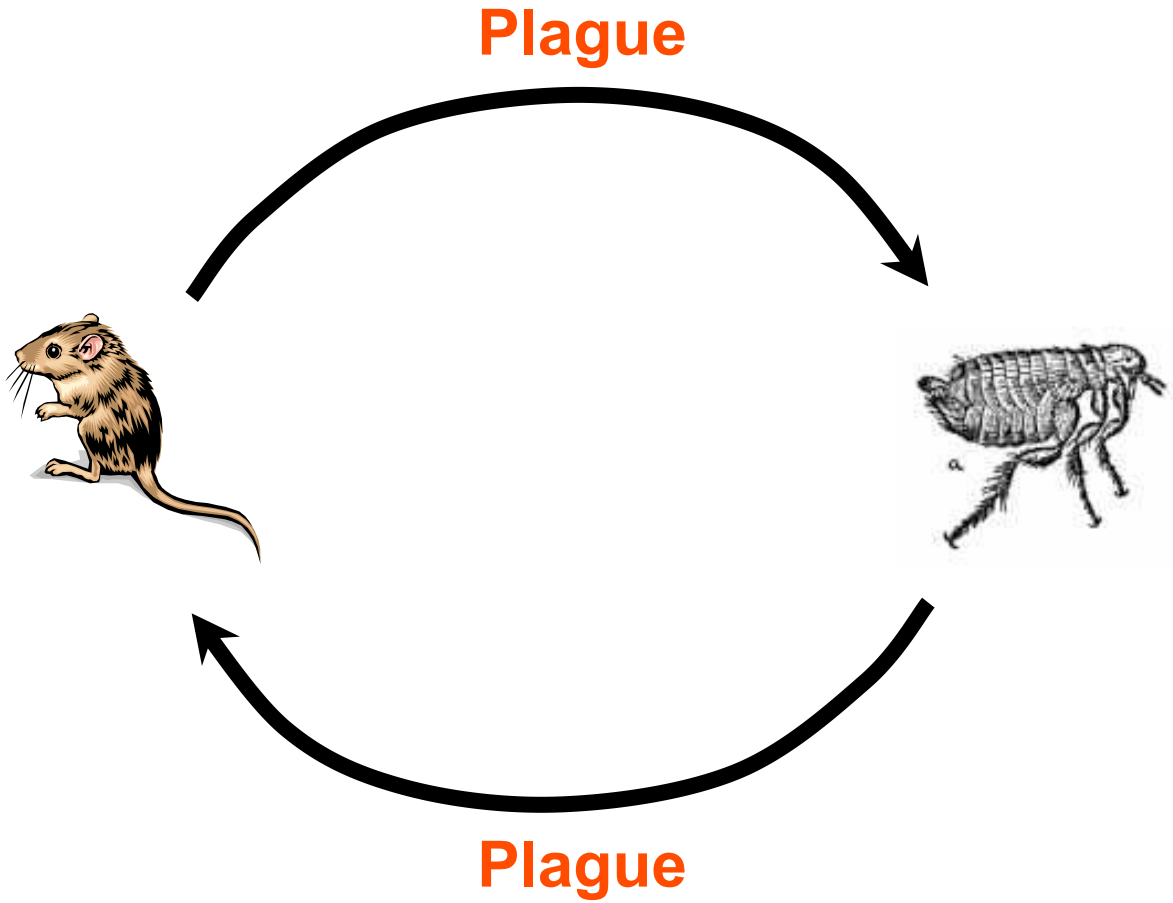
- > 30
- 20 - 30
- 10 - 19
- 5 - 9
- 1 - 4
- 0

264 Total Cases

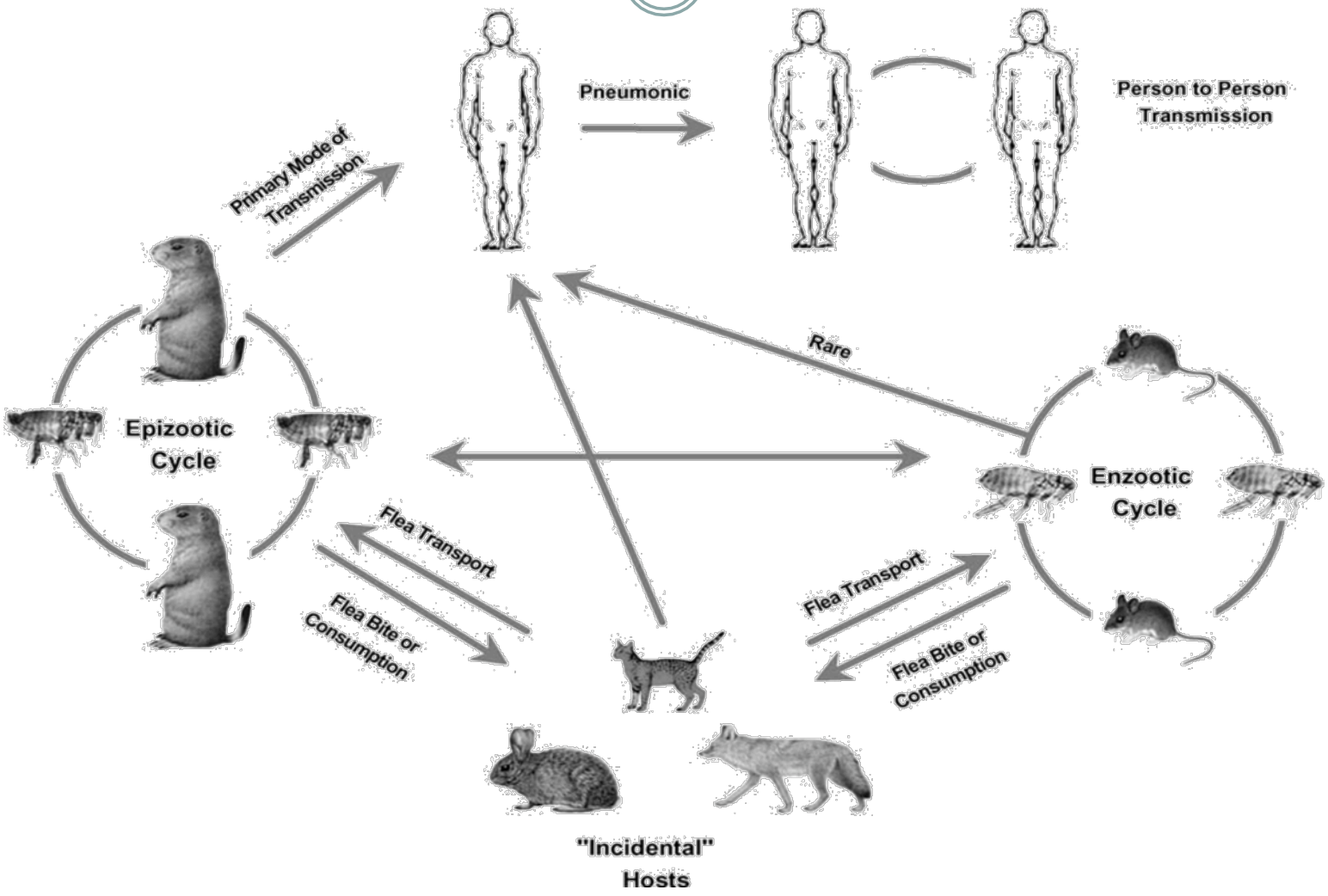
Human Plague by Onset Month
New Mexico, 1949–2011



Basic Plague Ecology

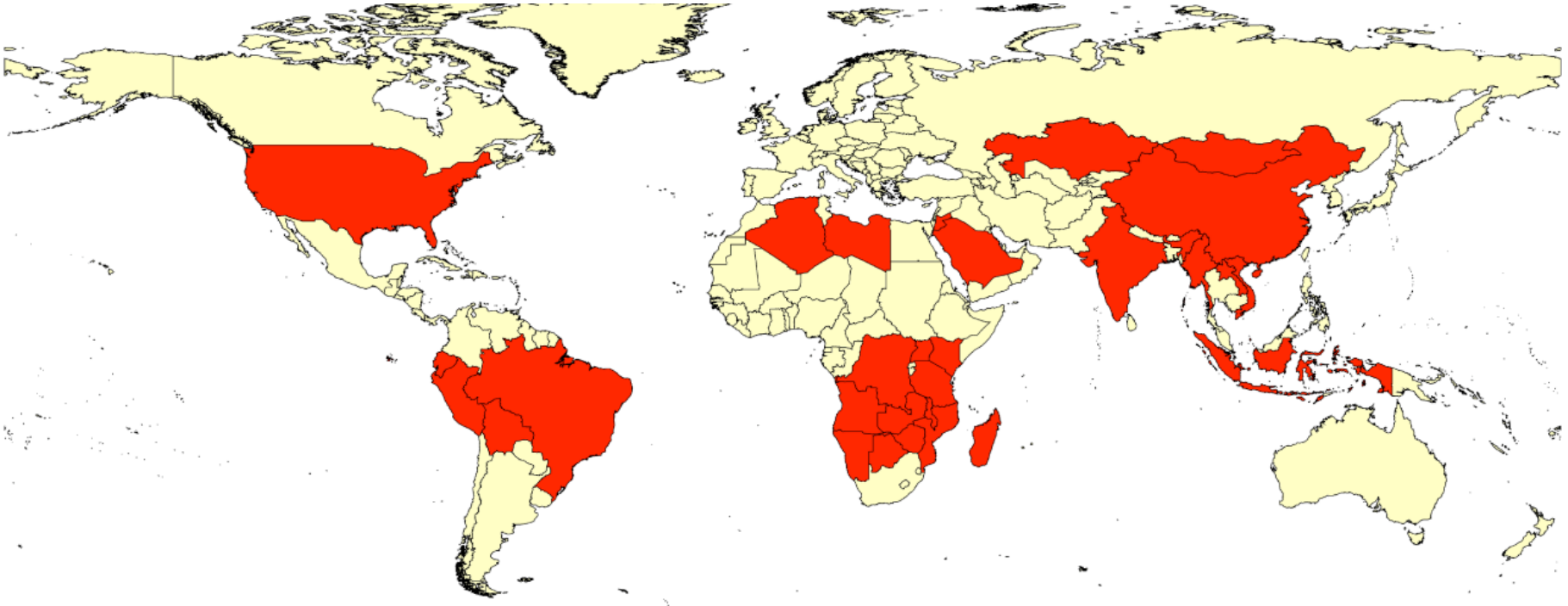


More Complex Plague Ecology



Gage and Kosoy. 2005. Annual Review of Entomology 50:505-528.

Plague is Now Globally Distributed



Stenseth *et al.* 2008. PLoS Medicine 5(1):e3

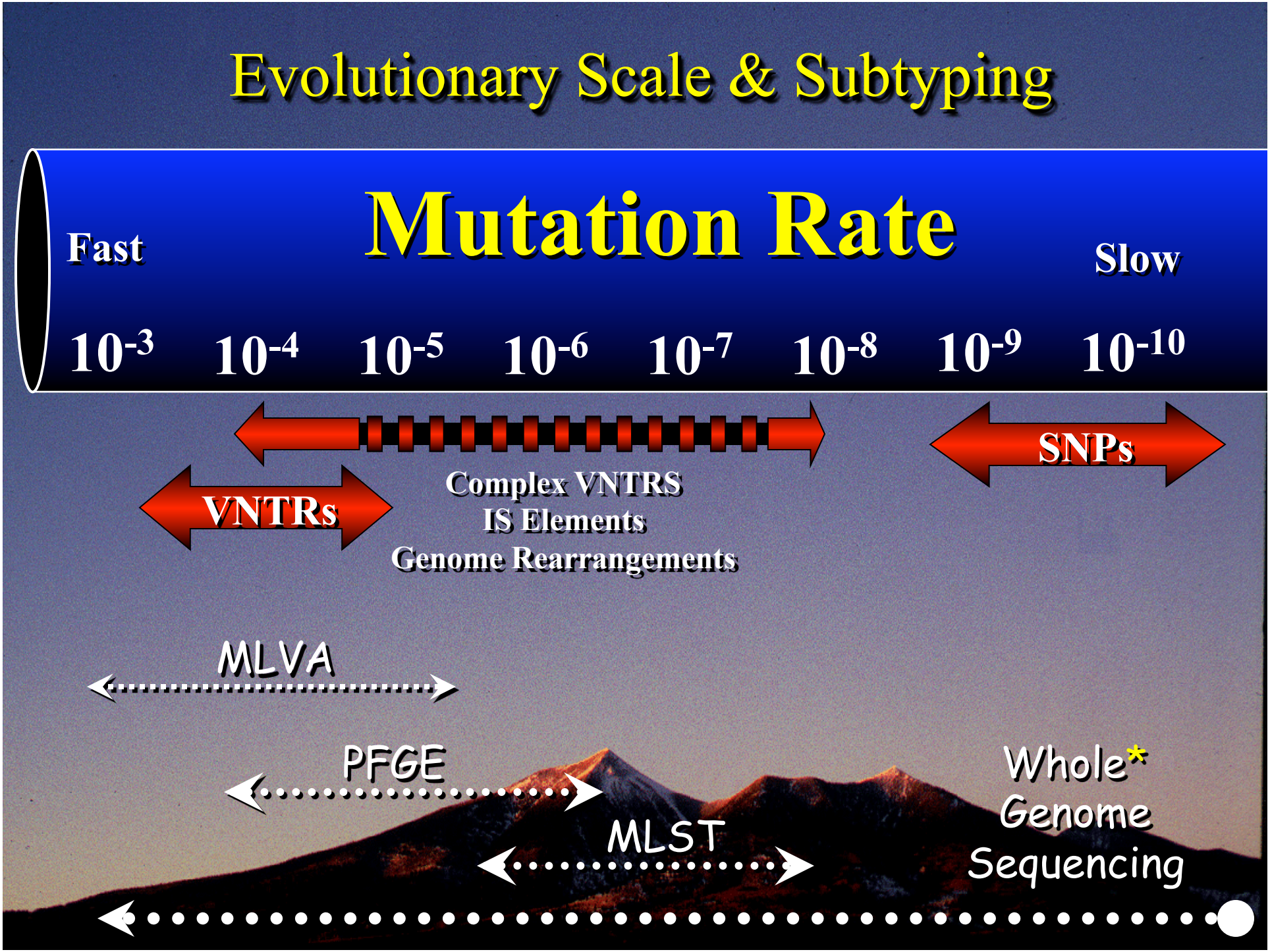
Three Plague Pandemics (Dogma)

- 1st Pandemic: *(antiqua biovar)*
  - 547-767 AD "Justinian plague"
  - North Africa, Europe, parts of Asia
  - Population losses estimated at 50-60%
- 2nd Pandemic: *(mediaevalis biovar)*
  - 1346-19th century
  - North Africa, Europe, parts of Asia
  - Black Death epidemic: 1347-1351
    - Est. 17-28 million killed, or ~30-40% of the European population
- 3rd Pandemic: *(orientalis biovar)*
  - 1855-present?
  - Global spread out of China: Africa, Americas, Australia, Europe
  - Millions of deaths worldwide
  - Control: improved hygiene (rat control) and antibiotics

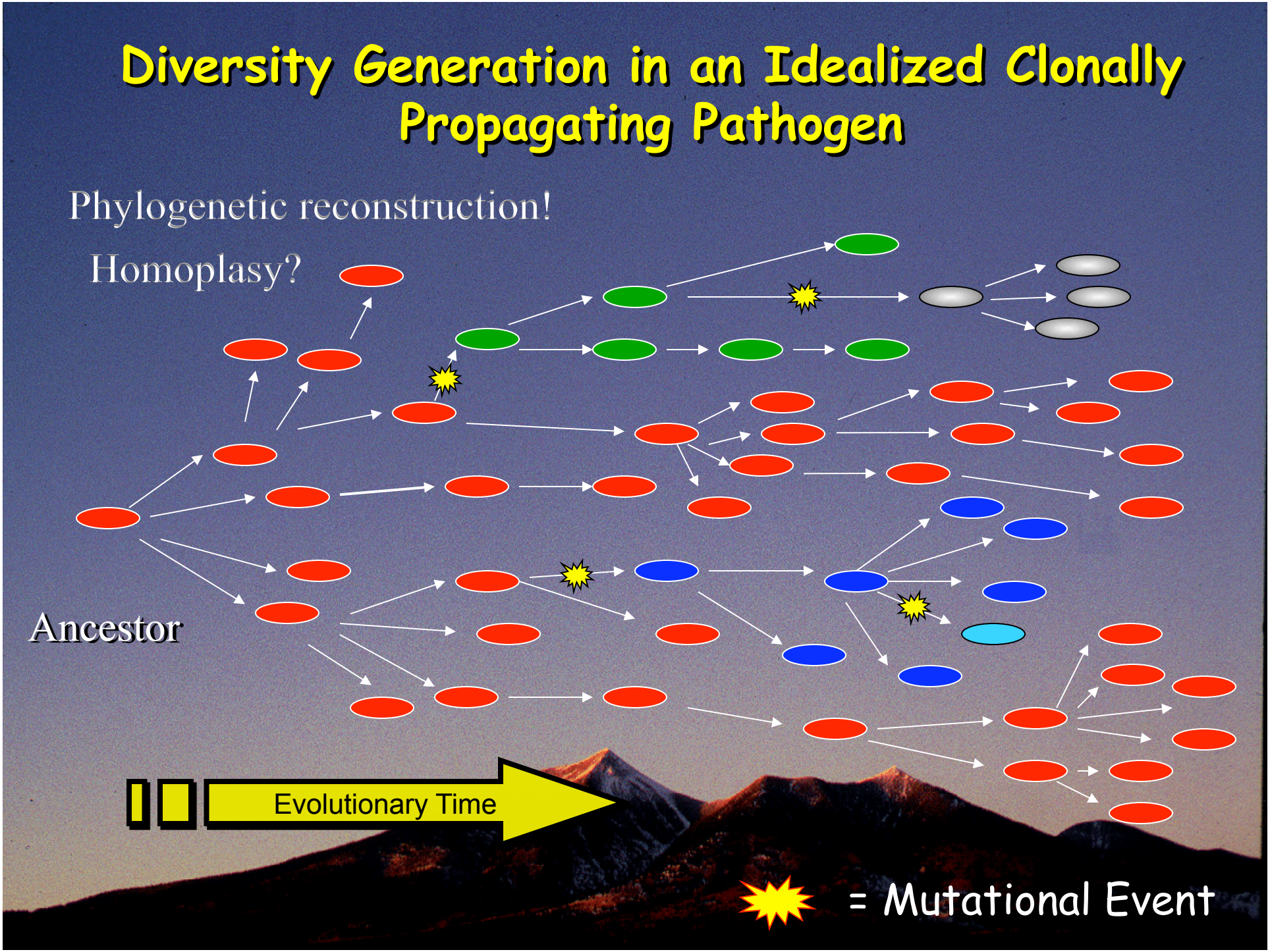
Devignat 1951

Perry and Fetherston. 1997. Clinical Microbiology Reviews 10:35-66.

Evolutionary Scale & Subtyping



Diversity Generation in an Idealized Clonally Propagating Pathogen



PNAS

Microevolution and history of the plague bacillus, *Yersinia pestis*

Mark Achtman*†, Giovanna Morelli*, Peixuan Zhu*‡, Thierry Wirth*§, Ines Diehl*, Barica Kusecek*, Amy J. Vogler¶, David M. Wagner¶, Christopher J. Allender¶, W. Ryan Easterday¶, Viviane Chenal-Francisque‖, Patricia Worsham**, Nicholas R. Thomson††, Julian Parkhill††, Luther E. Lindler‡‡§§, Elisabeth Carniel‖, and Paul Keim¶,¶¶

*Department of Molecular Biology, Max-Planck Institut für Infektionsbiologie, D-10117 Berlin, Germany; ¶Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011-5640; ‖Yersinia Research Unit, Institut Pasteur, 75724 Paris Cedex 15, France; **U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD 21702-5011; ††The Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, United Kingdom; ‡‡National Biodefense Analysis and Countermeasures Center, Frederick, MD 21703; §§Department of Bacterial Diseases, Walter Reed Army Institute of Research, Silver Spring, MD 20910; and ¶¶Translational Research Institute, Phoenix, AZ 85004

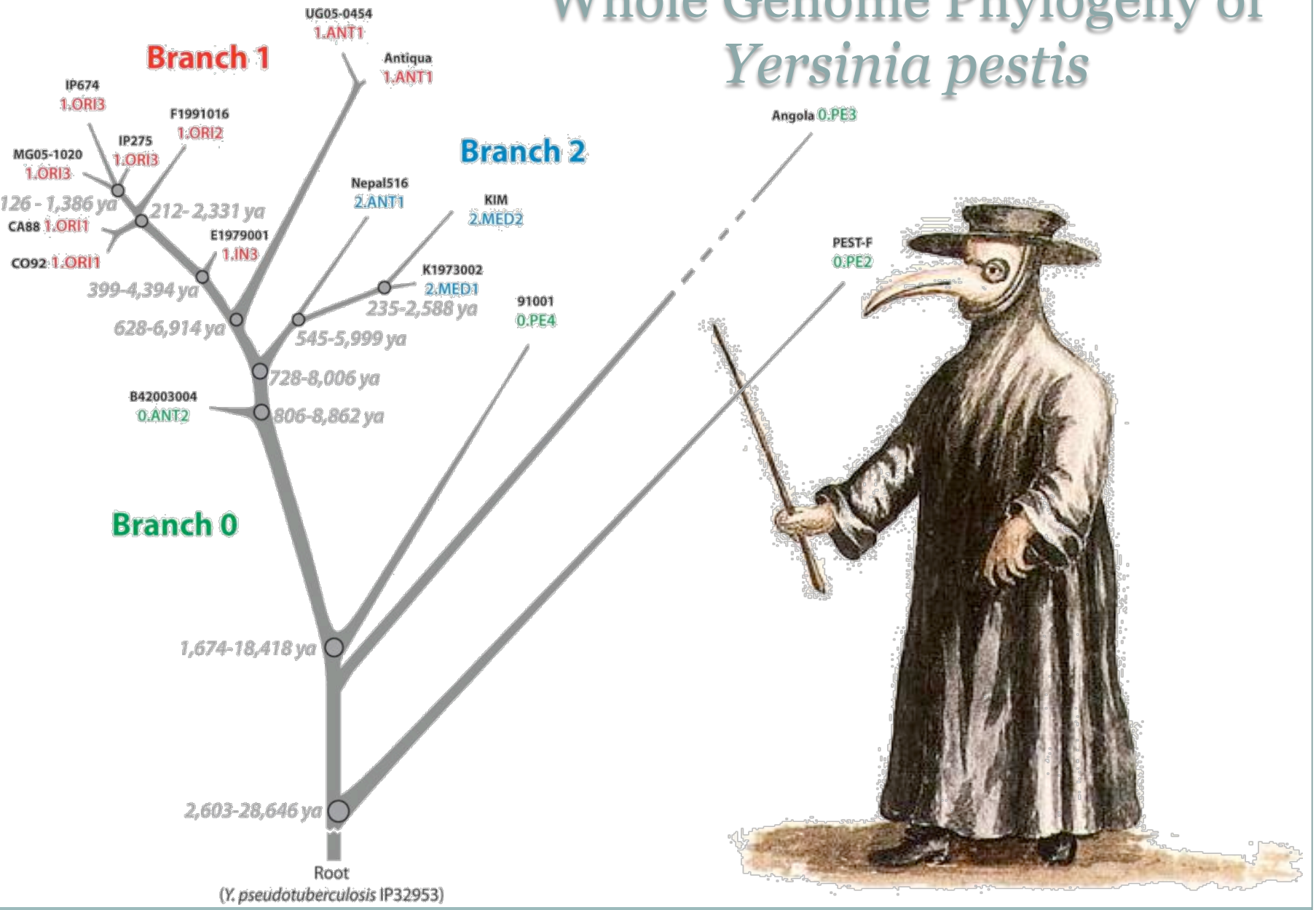
nature genetics

Yersinia pestis genome sequencing identifies patterns of global phylogenetic diversity

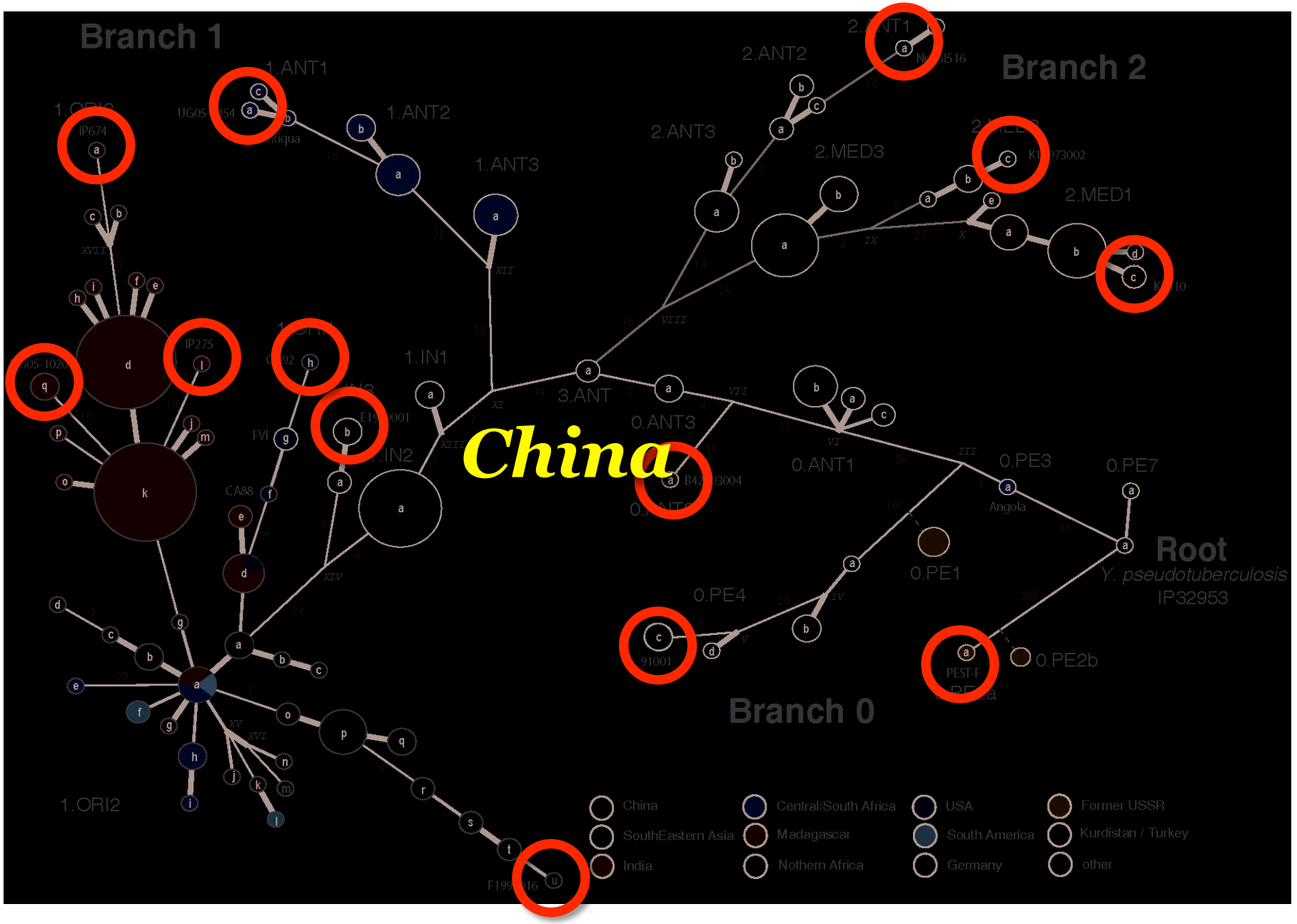
Giovanna Morelli[1,16], Yajun Song[2,3,16], Camila J Mazzoni[1,3,16], Mark Eppinger[4,16], Philippe Roumagnac[1,5], David M Wagner[6], Mirjam Feldkamp[1], Barica Kusecek[1], Amy J Vogler[6], Yanjun Li[2], Yujun Cui[2], Nicholas R Thomson[7], Thibaut Jombart[8], Raphael Leblois[9], Peter Lichtner[10], Lila Rahalison[11], Jeannine M Petersen[12], Francois Balloux[8], Paul Keim[6,13], Thierry Wirth[1,9], Jacques Ravel[4], Ruifu Yang[2], Elisabeth Carniel[14] & Mark Achtman[1,3,15]

Achtman *et al.* 2004. PNAS; Morelli *et al.* 2010. Nature Genetics.

Whole Genome Phylogeny of *Yersinia pestis*

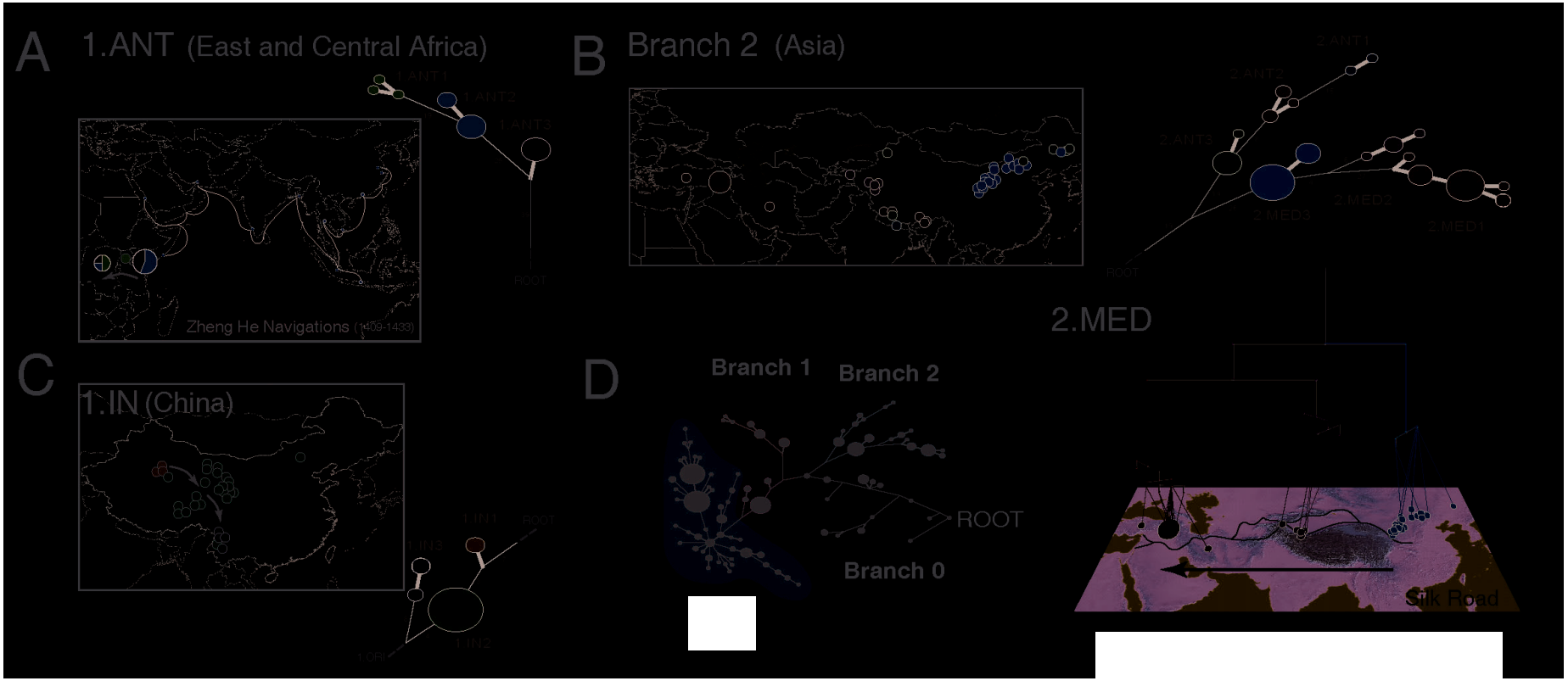


Morelli *et al.* 2010. Nature Genetics 42:1140-1143.



Morelli *et al.* 2010. Nature Genetics 42:1140-1143.

Plague Out of China Again and Again



Morelli *et al.* 2010. Nature Genetics 42:1140-1143.

OPEN ACCESS Freely available online

PLoS PATHOGENS

Distinct Clones of *Yersinia pestis* Caused the Black Death

Stephanie Haensch[1], Raffaella Bianucci[2,3], Michel Signoli[3,4], Minoarisoa Rajerison[5], Michael Schultz[6], Sacha Kacki[7,8], Marco Vermunt[9], Darlene A. Weston[10,11,12], Derek Hurst[13], Mark Achtman[14], Elisabeth Carniel[15], Barbara Bramanti[1]*

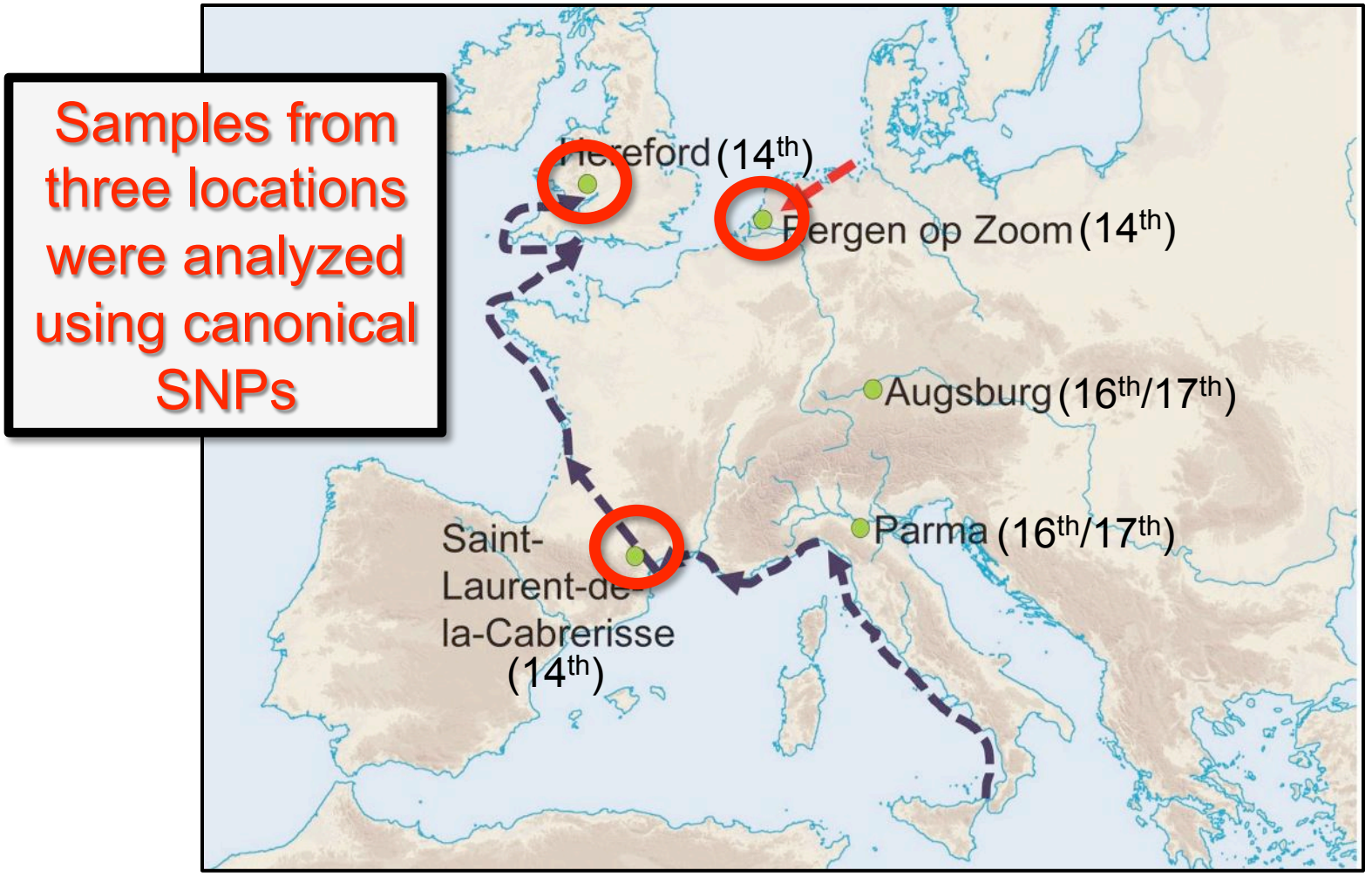
1 Institute for Anthropology, Johannes Gutenberg University, Mainz, Germany, **2** Laboratory of Criminalistic Sciences Department of Anatomy, Pharmacology and Legal Medicine, University of Turin, Turin, Italy, **3** Unité d'Anthropologie Bioculturelle, Faculté de Medecine, University of Mediterranean-CNRS-EFS, Marseille, France, **4** Centre d'Études Préhistoire, Antiquité, Moyen-âge, UMR 6130 CNRS–250 University of Nice, Valbonne, France, **5** Center for Plague, Institute Pasteur de Madagascar, World Health Organization Collaborating, Antananarivo, Madagascar, **6** Department of Anatomy and Embryology Medical Faculty, Georg-August University, Göttingen, Germany, **7** Inrap, Villeneuve-d'Ascq Archaeological Center, Villeneuve-d'Ascq, France, **8** Laboratoire d'Anthropologie des Populations du Passé, Université Bordeaux 1, Talence, France, **9** Department of Monuments and Archaeology, Municipality of Bergen op Zoom, Bergen op Zoom, The Netherlands, **10** Barge's Anthropologica, Department of Anatomy and Embryology, Leiden University Medical Center, Leiden, The Netherlands, **11** Division of Archaeological Sciences, University of Bradford, Bradford, West Yorkshire, United Kingdom, **12** Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany, **13** Worcestershire Historic Environment and Archaeology Service, Worcestershire County Council, Worcester, United Kingdom, **14** Environmental Research Institute, University College Cork, Cork, Ireland, **15** Yersinia Research Unit, Institut Pasteur, Paris, France

Abstract

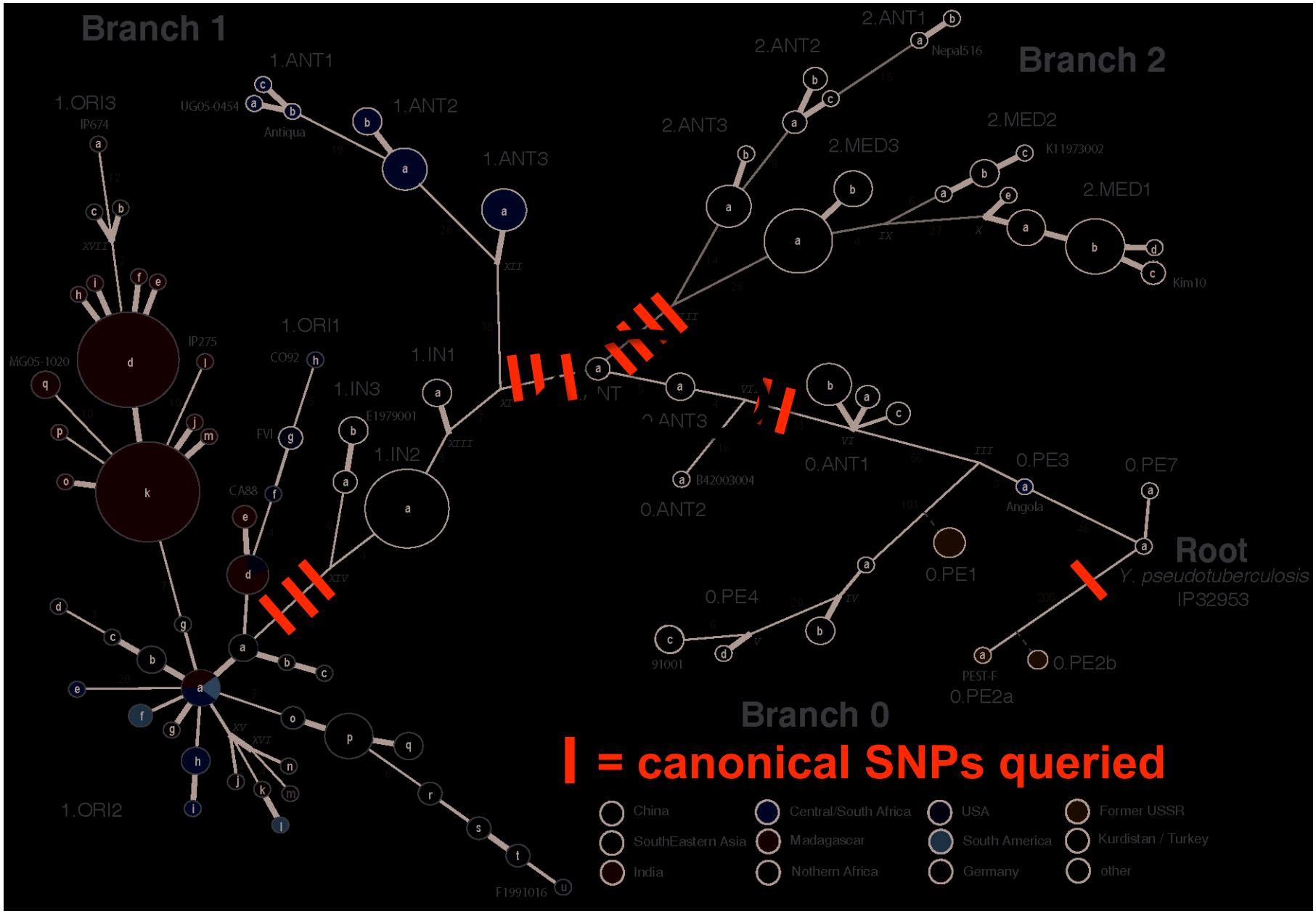
From AD 1347 to AD 1353, the Black Death killed tens of millions of people in Europe, leaving misery and devastation in its wake, with successive epidemics ravaging the continent until the 18th century. The etiology of this disease has remained highly controversial, ranging from claims based on genetics and the historical descriptions of symptoms that it was caused by *Yersinia pestis* to conclusions that it must have been caused by other pathogens. It has also been disputed whether plague had the same etiology in northern and southern Europe. Here we identified DNA and protein signatures specific for *Y. pestis* in human skeletons from mass graves in northern, central and southern Europe that were associated archaeologically with the Black Death and subsequent resurgences. We confirm that *Y. pestis* caused the Black Death and later epidemics on the entire European continent over the course of four centuries. Furthermore, on the basis of 17 single nucleotide polymorphisms plus the absence of a deletion in *glpD* gene, our aDNA results identified two previously unknown but related clades of *Y. pestis* associated with distinct medieval mass graves. These findings suggest that plague was imported to Europe on two or more occasions, each following a distinct route. These two clades are ancestral to modern isolates of *Y. pestis* biovars Orientalis and Medievalis. Our results clarify the etiology of the Black Death and provide a paradigm for a detailed historical reconstruction of the infection routes followed by this disease.

Haensch *et al.* 2010. PLoS Pathogens 6(10):e1001134

2nd Plague Pandemic: 14th – 18th Centuries



Haensch *et al.* 2010. PLoS Pathogens 6(10):e1001134

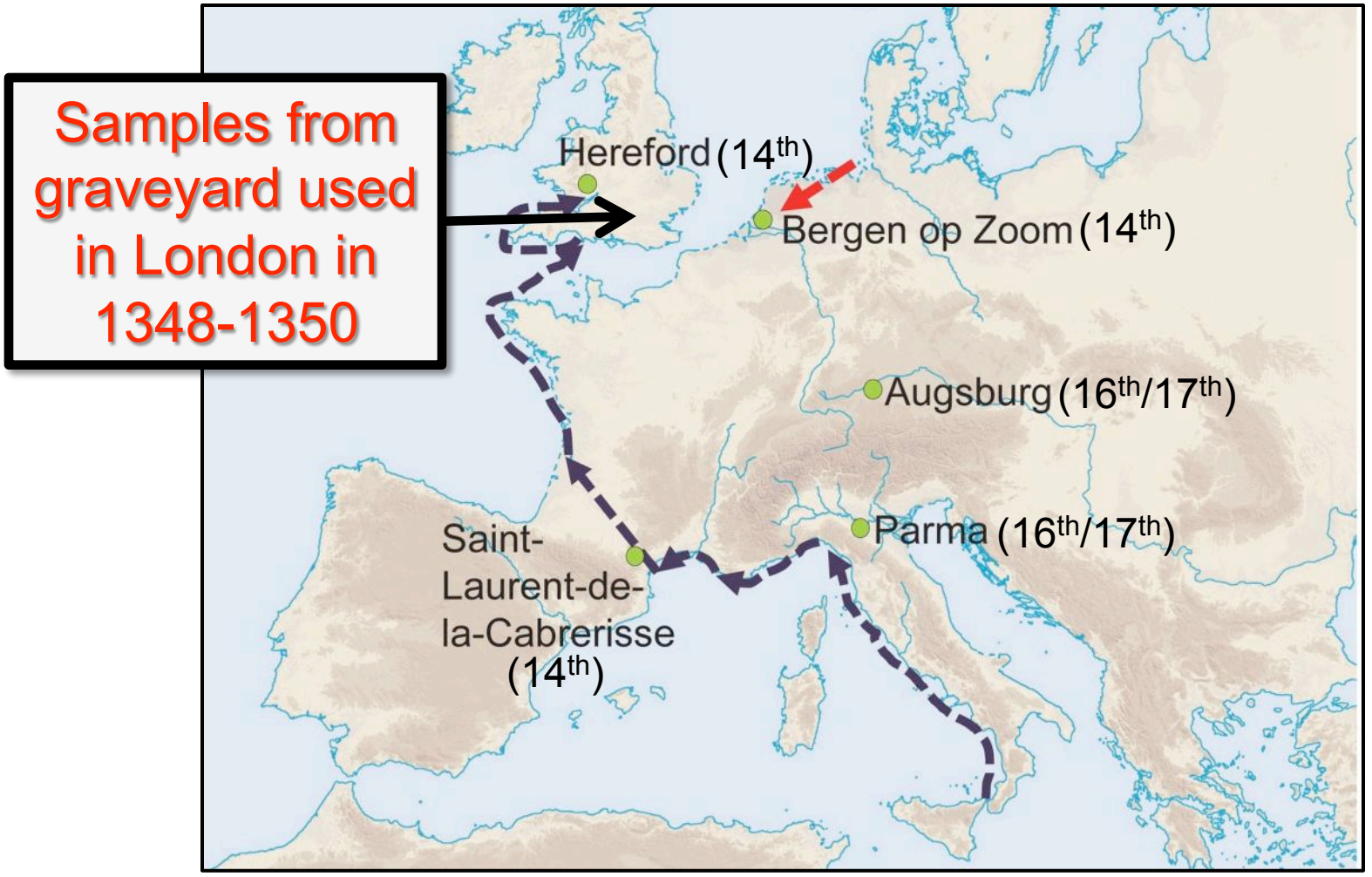


Haensch *et al.* 2010; Morelli *et al.* 2010

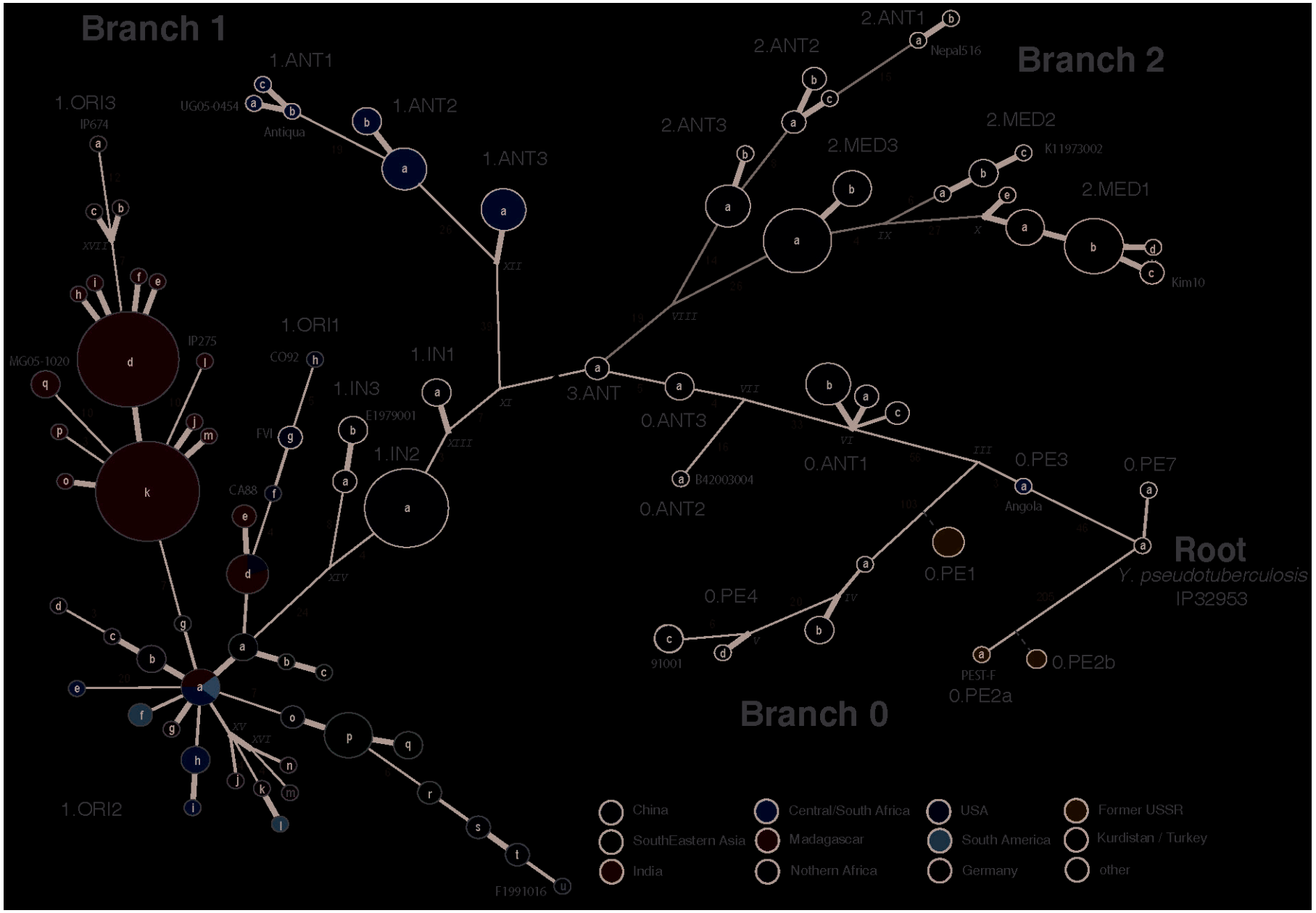

LETTER
doi:10.1038/nature10549
A draft genome of Yersinia pestis from victims of the Black Death
Kirsten I. Bos[1]*, Verena J. Schuenemann[2]*, G. Brian Golding[3], Hernán A. Burbano[4], Nicholas Waglechner[5], Brian K. Coombes[5], Joseph B. McPhee[5], Sharon N. DeWitte[6,7], Matthias Meyer[4], Sarah Schmedes[8], James Wood[9], David J. D. Earn[5,10], D. Ann Herring[11], Peter Bauer[12], Hendrik N. Poinar[1,3,5] & Johannes Krause[2,12]

NATURE 2011

2nd Plague Pandemic: 14th – 18th Centuries



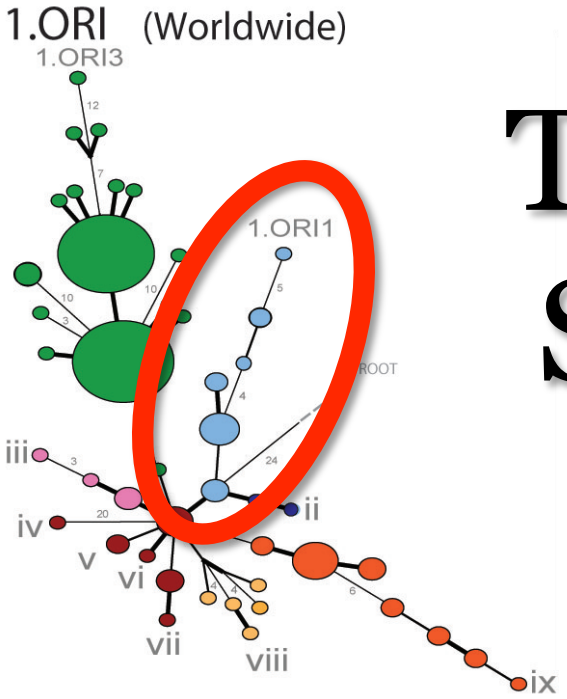
Bos *et al.* 2011. Nature 478:506-510

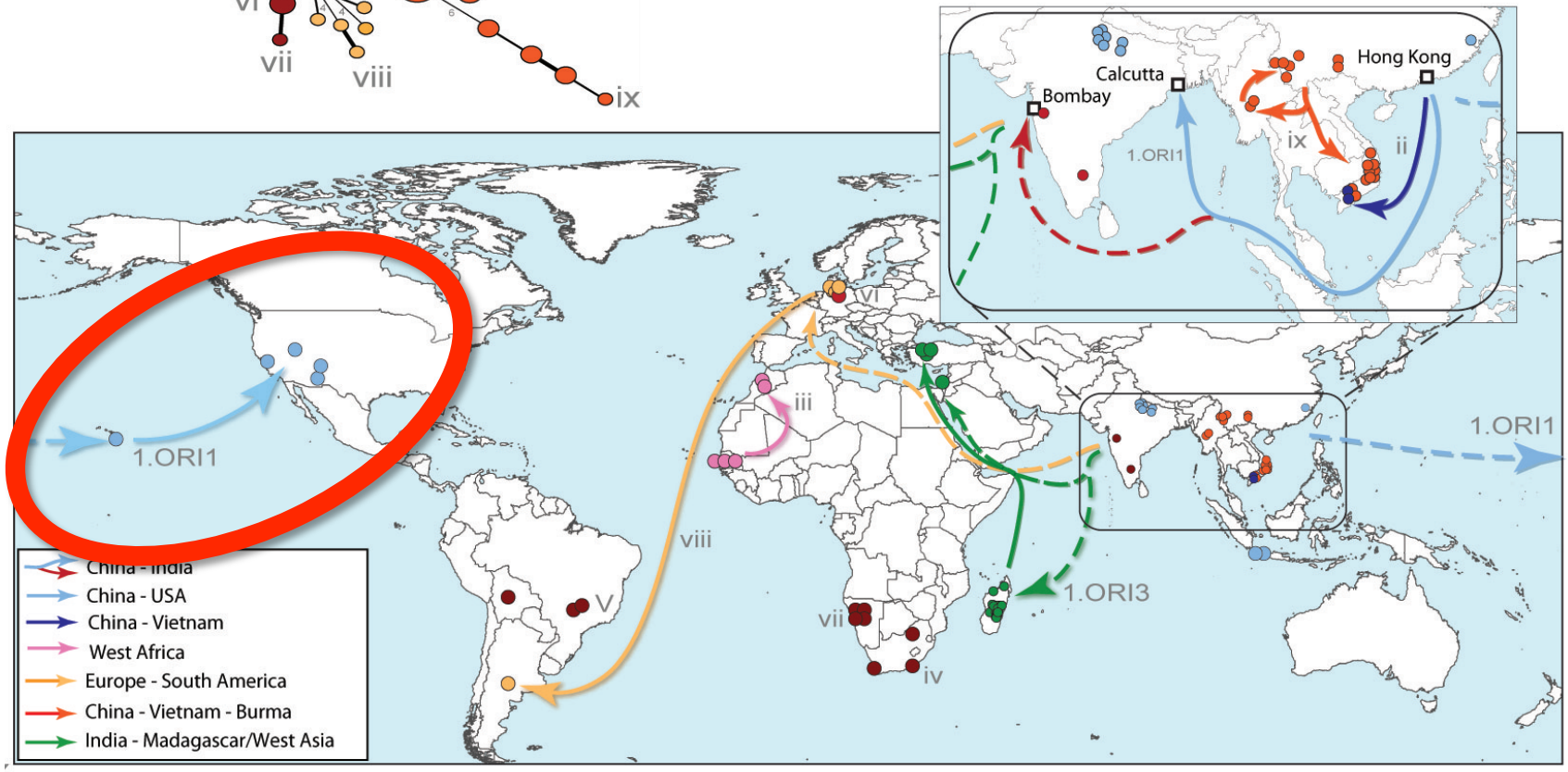


Bos *et al.* 2011; Morelli *et al.* 2010

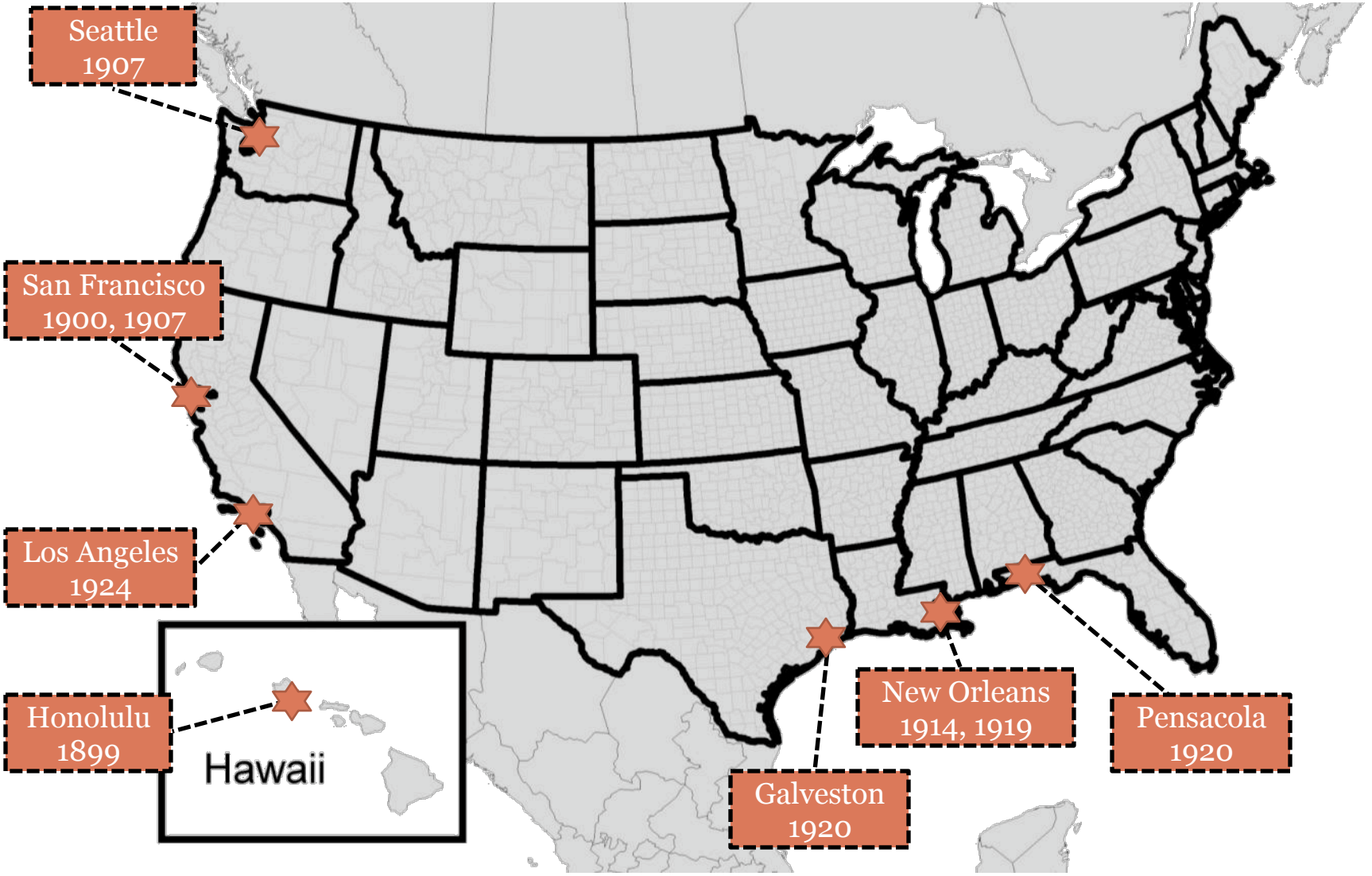
Third Pandemic: Spread of 1.ORI

Morelli *et al.* 2010. Nature Genetics.



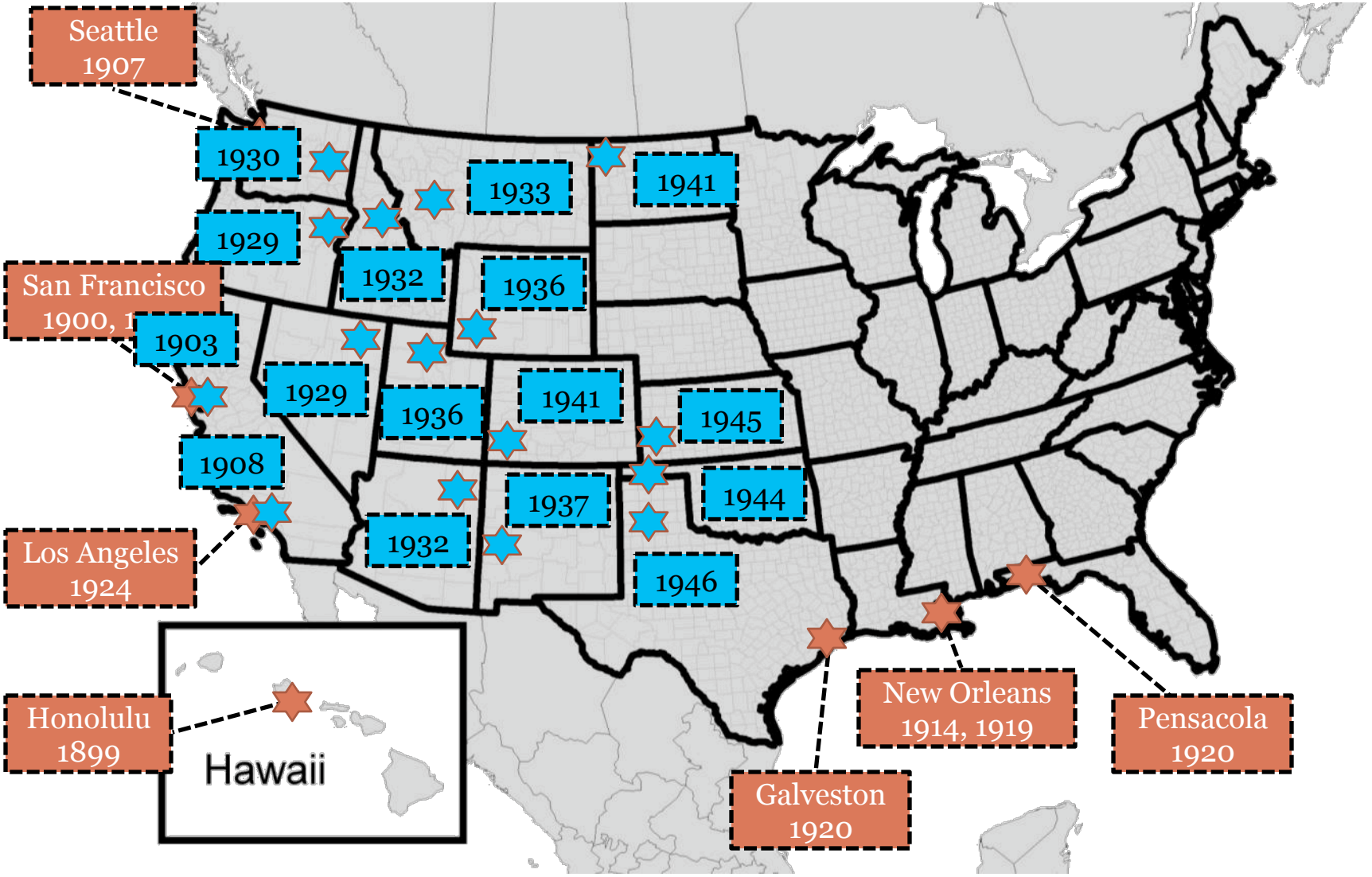


Rat-borne Plague in Multiple Port Cities



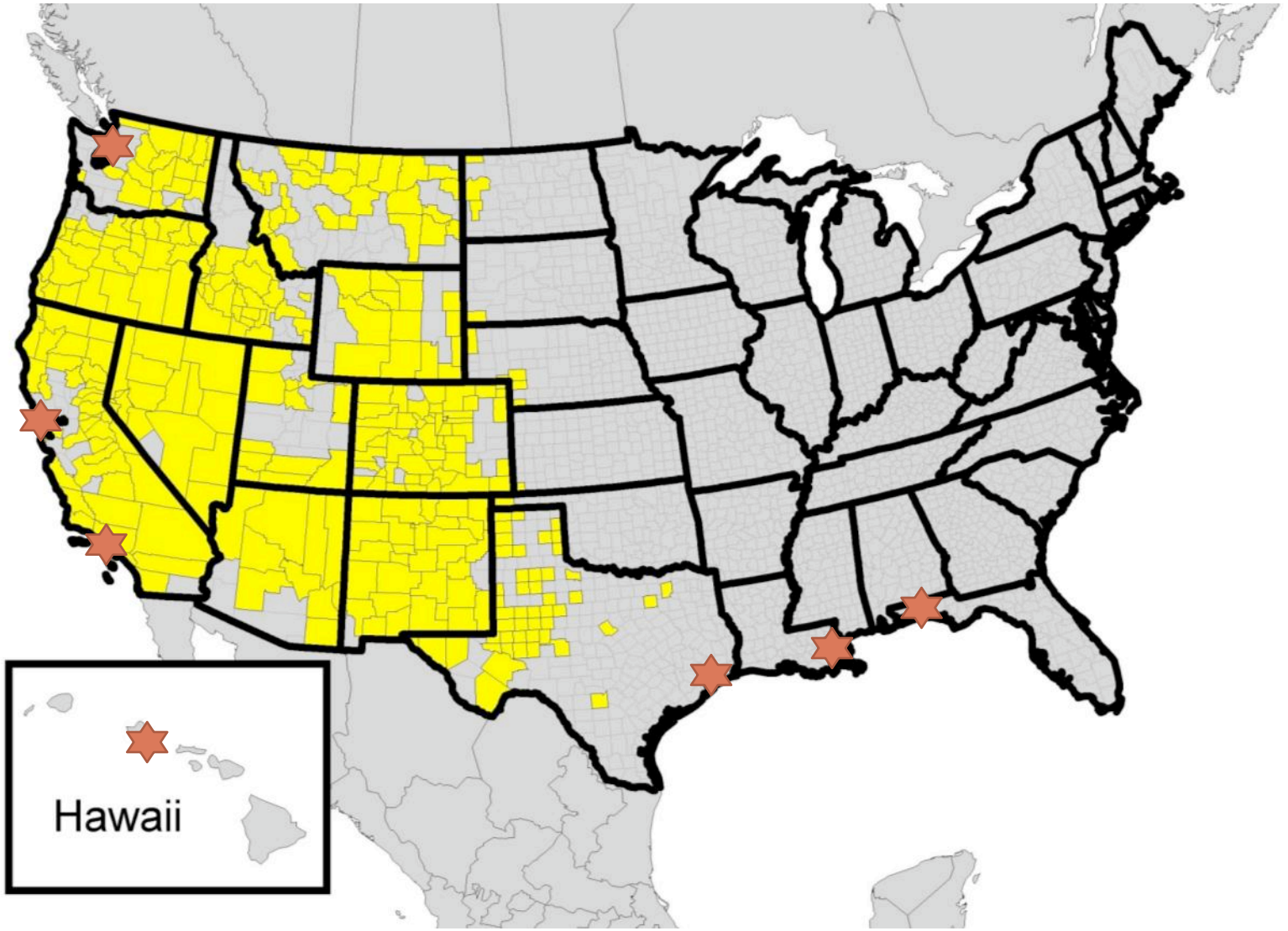
Eskey and Haas, 1940; Pollitzer 1951; Link 1955

Plague in Native Ground Squirrels



Eskey and Haas, 1940; Pollitzer 1951; Link 1955

Plague is Now Endemic in the Western US

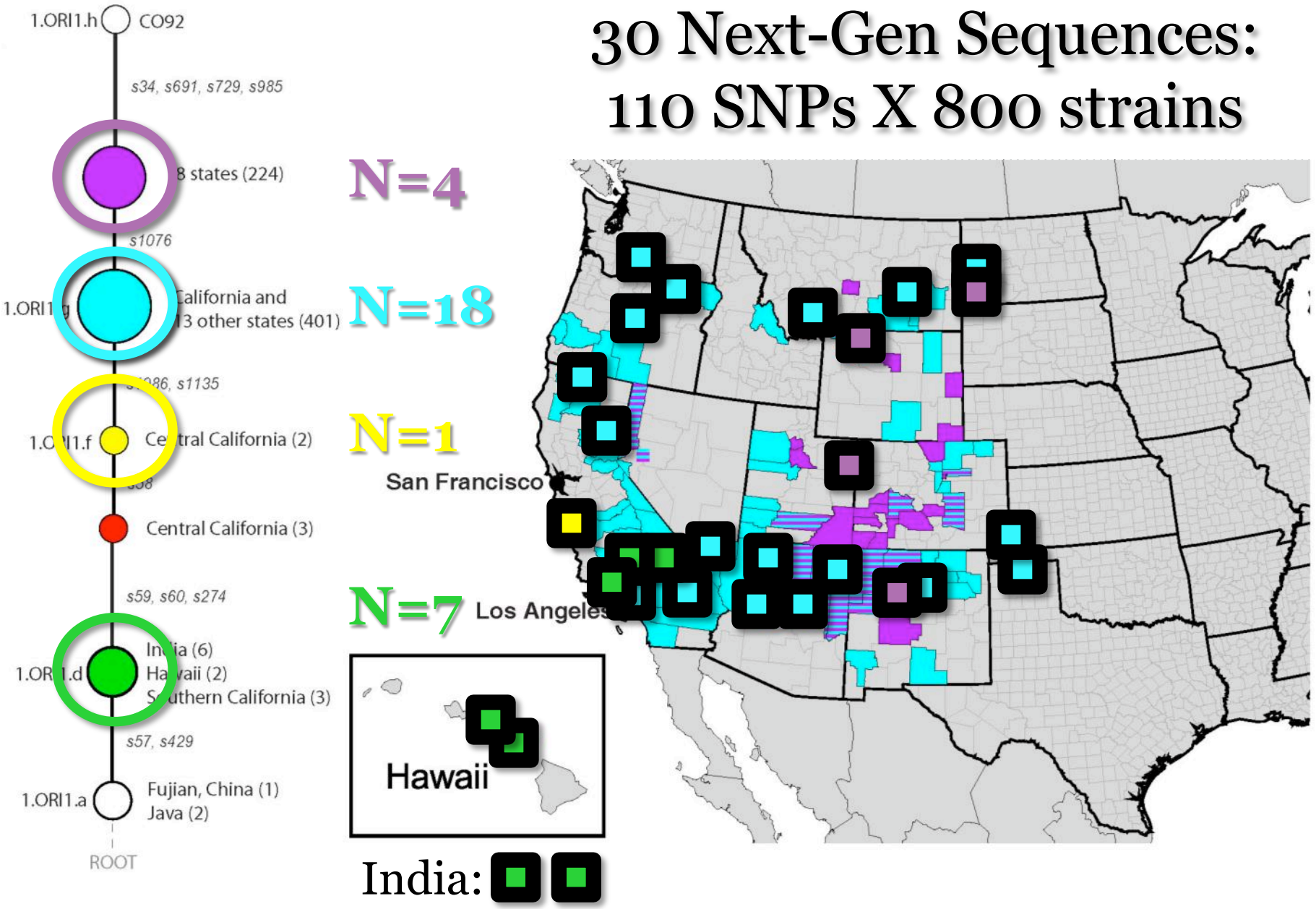


CDC occurrence data for 1970-2000 as compiled by Cully and Williams 2001

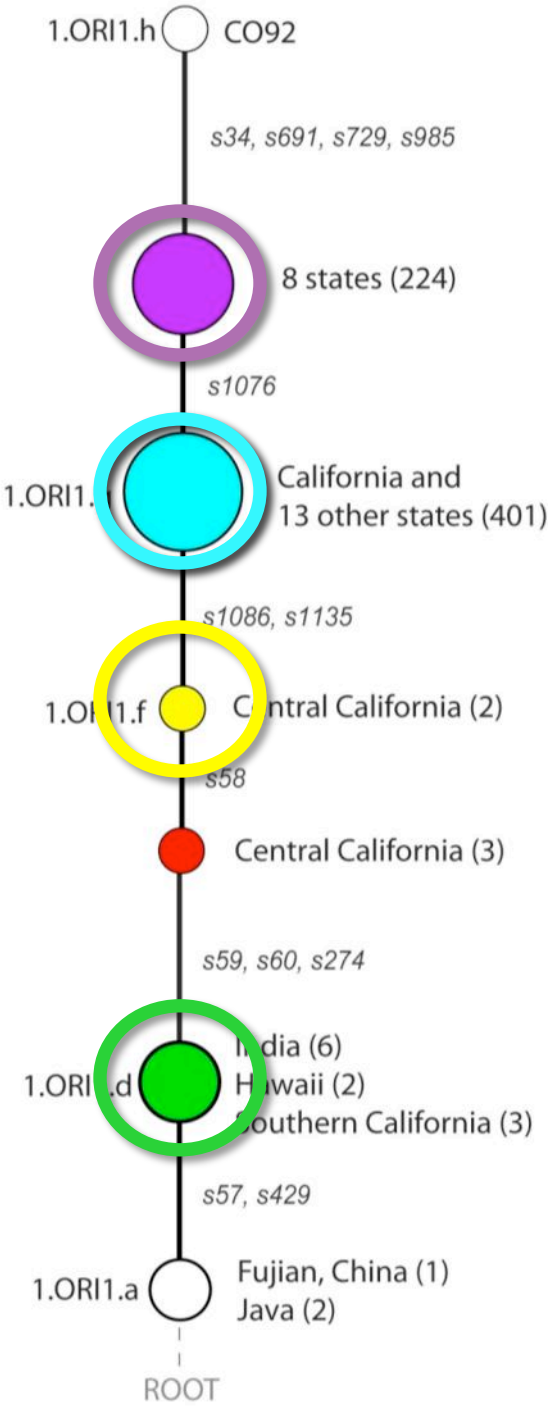
Questions

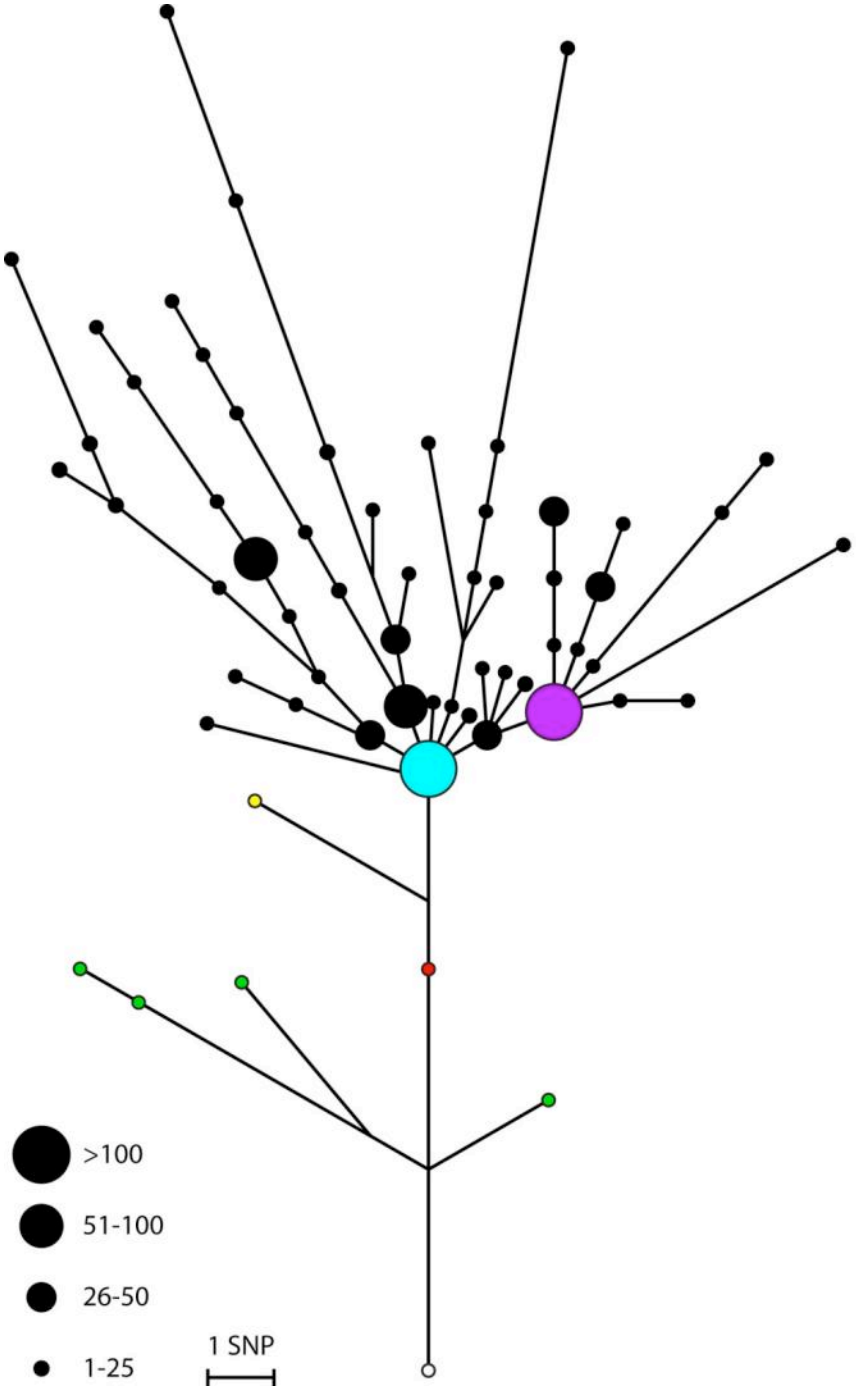
- How many times did plague cross over from non-native rodents to native rodents in the United States?
  - In other words, how many introductions were there in the United States?
- What is the population structure of *Yersinia pestis* in the United States?
  - Single wave of dominant clone? Multiple waves?

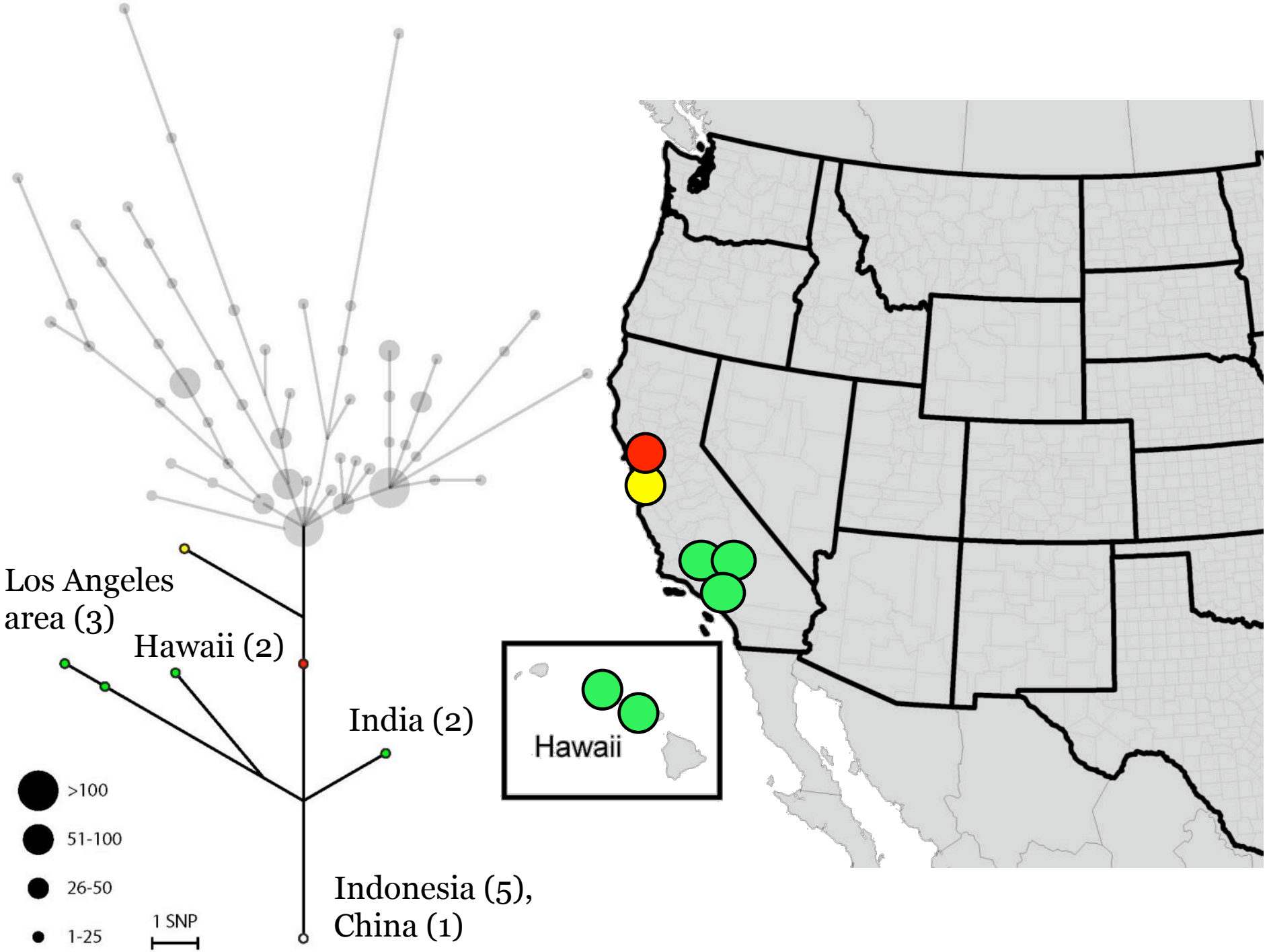
30 Next-Gen Sequences: 110 SNPs X 800 strains



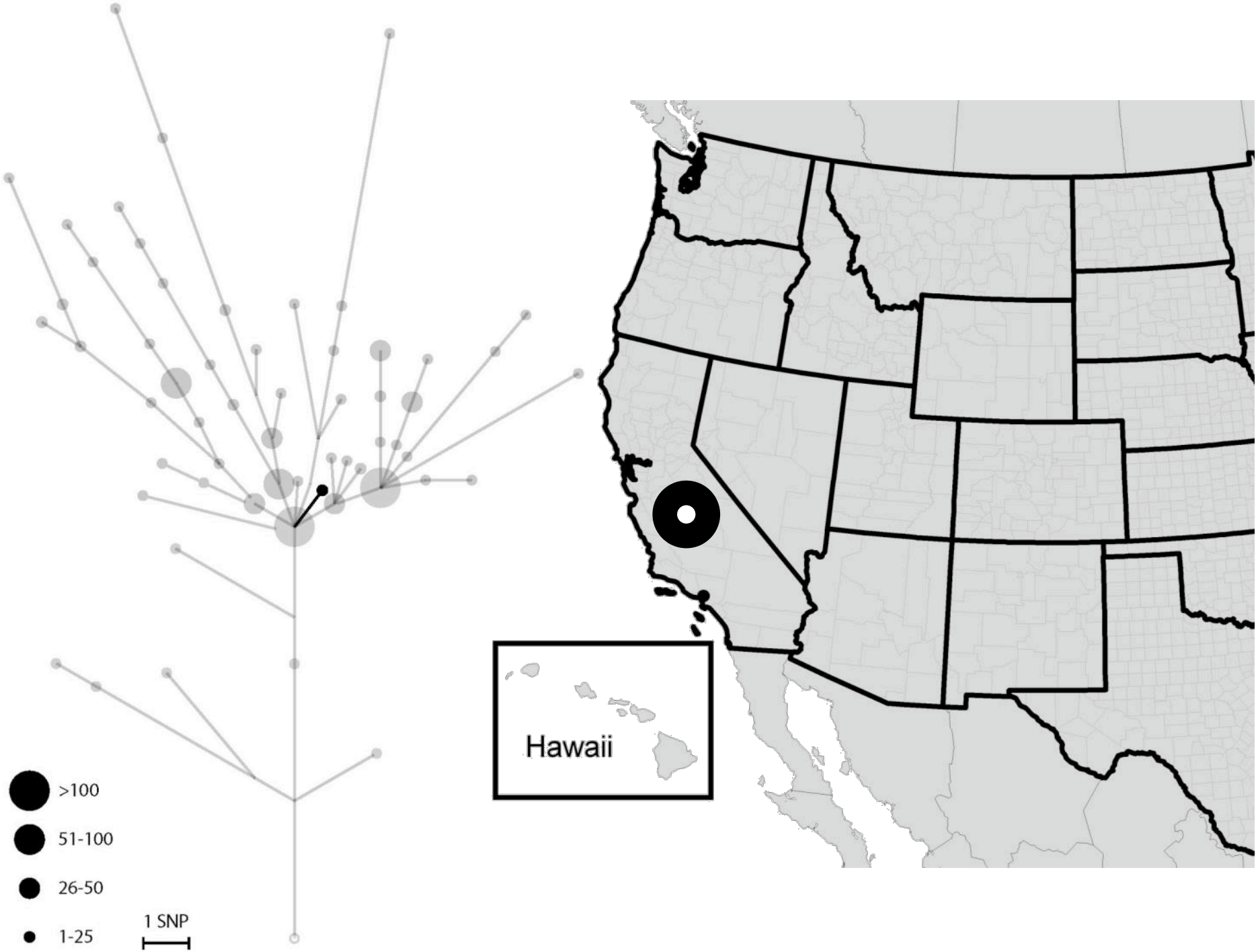
Morelli *et al.* 2010. Nature Genetics.

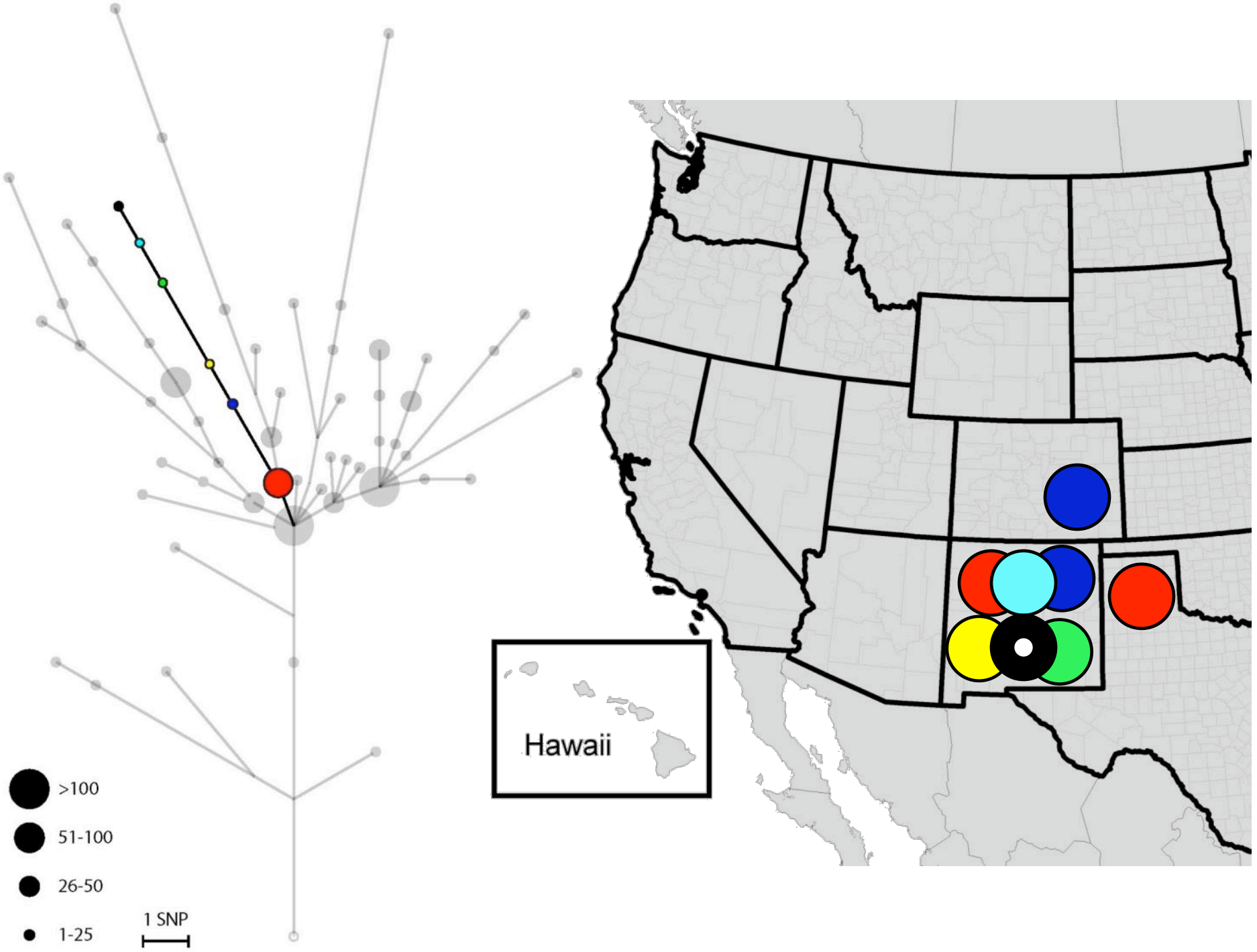

1.ORI1.h
CO92
s34, s691, s729, s985
8 states (224)
s1076
1.ORI1.
California and
13 other states (401)
s1086, s1135
1.OR 1.f
C ntral California (2)
s58
Central California (3)
s59, s60, s274
1.ORI .d
I dia (6)
H waii (2)
outhern California (3)
s57, s429
1.ORI1.a
Fujian, China (1)
Java (2)
ROOT

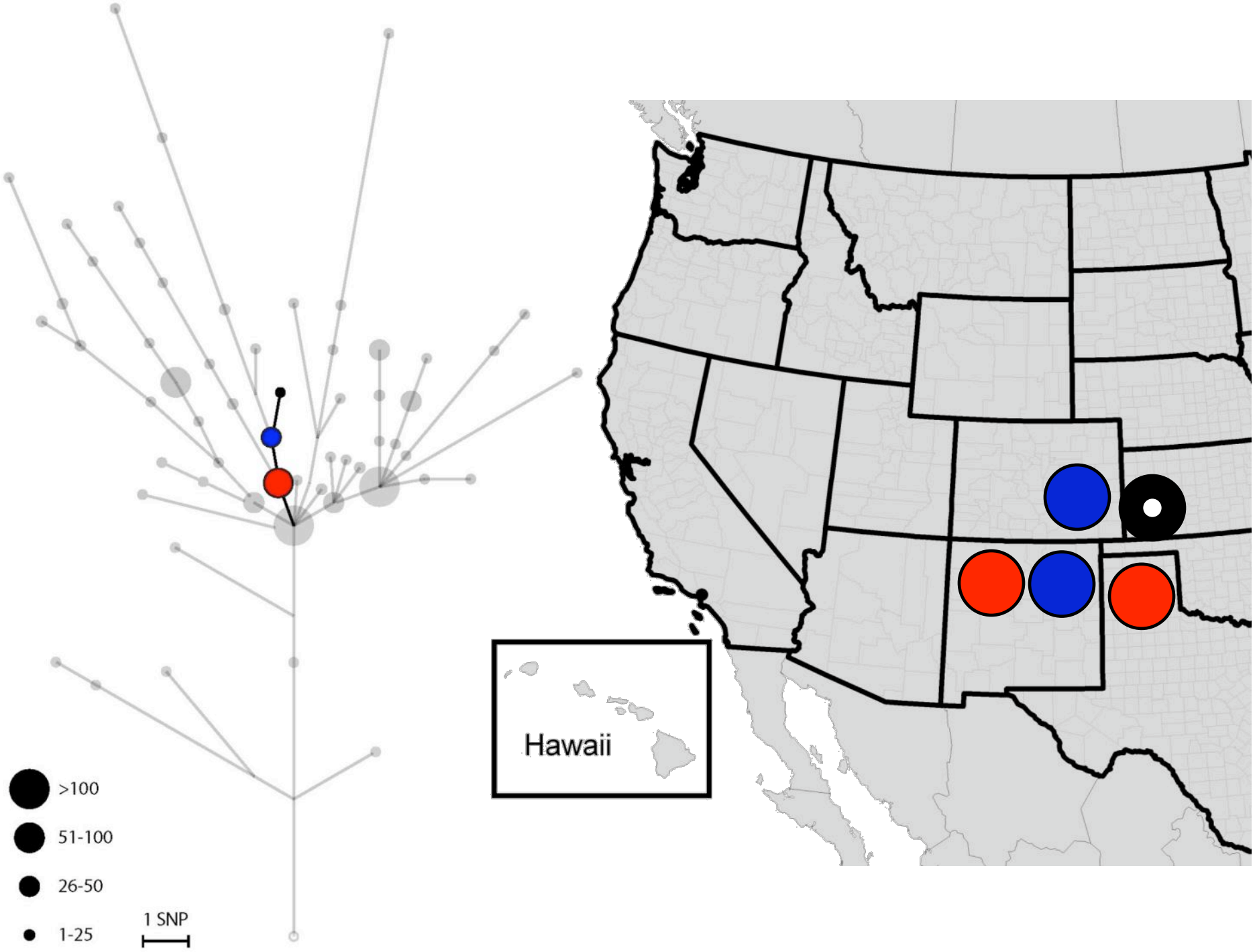

>100
51-100
26-50
1-25
1 SNP

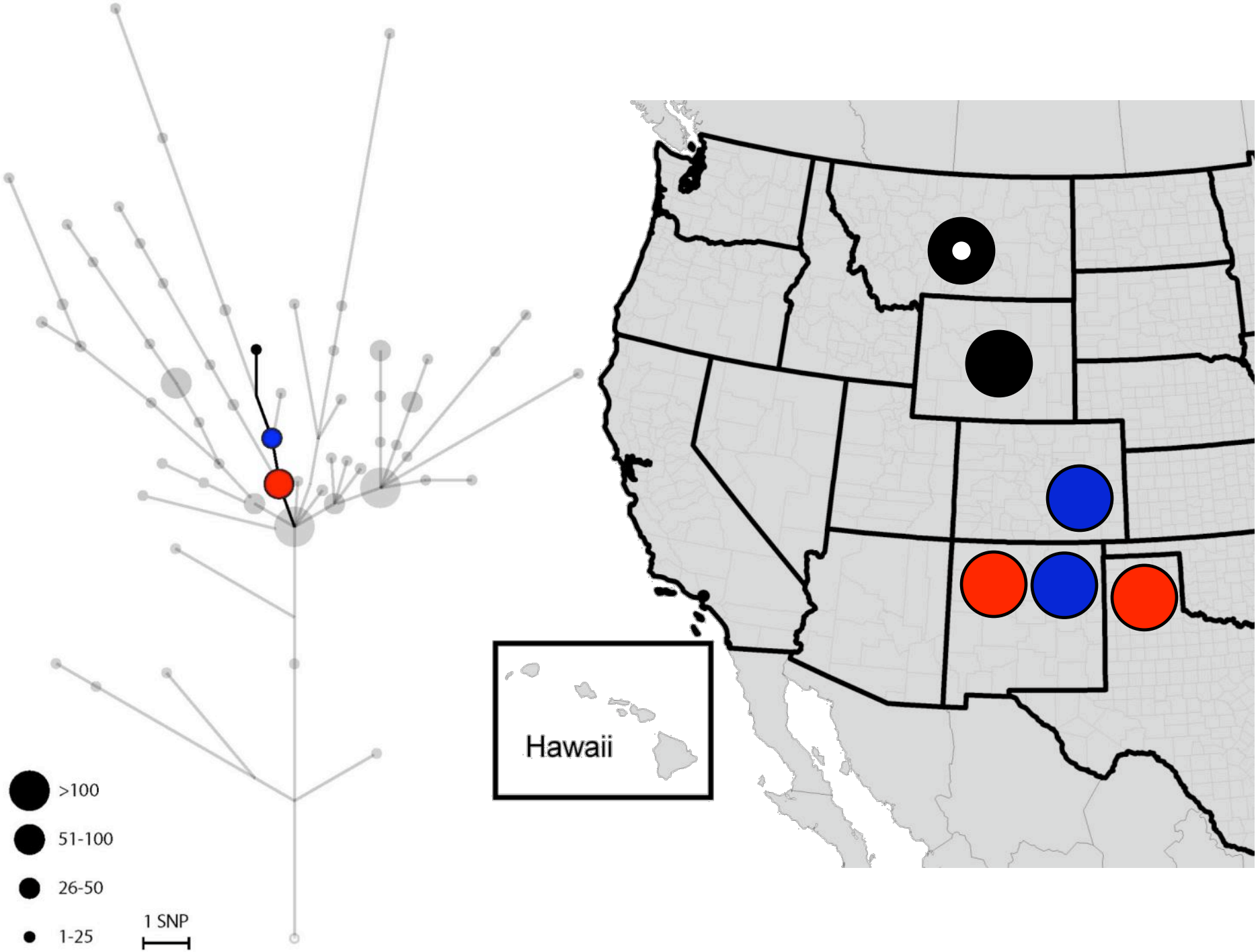

Los Angeles area (3)
Hawaii (2)
India (2)
Indonesia (5), China (1)
>100
51-100
26-50
1-25
1 SNP
Hawaii

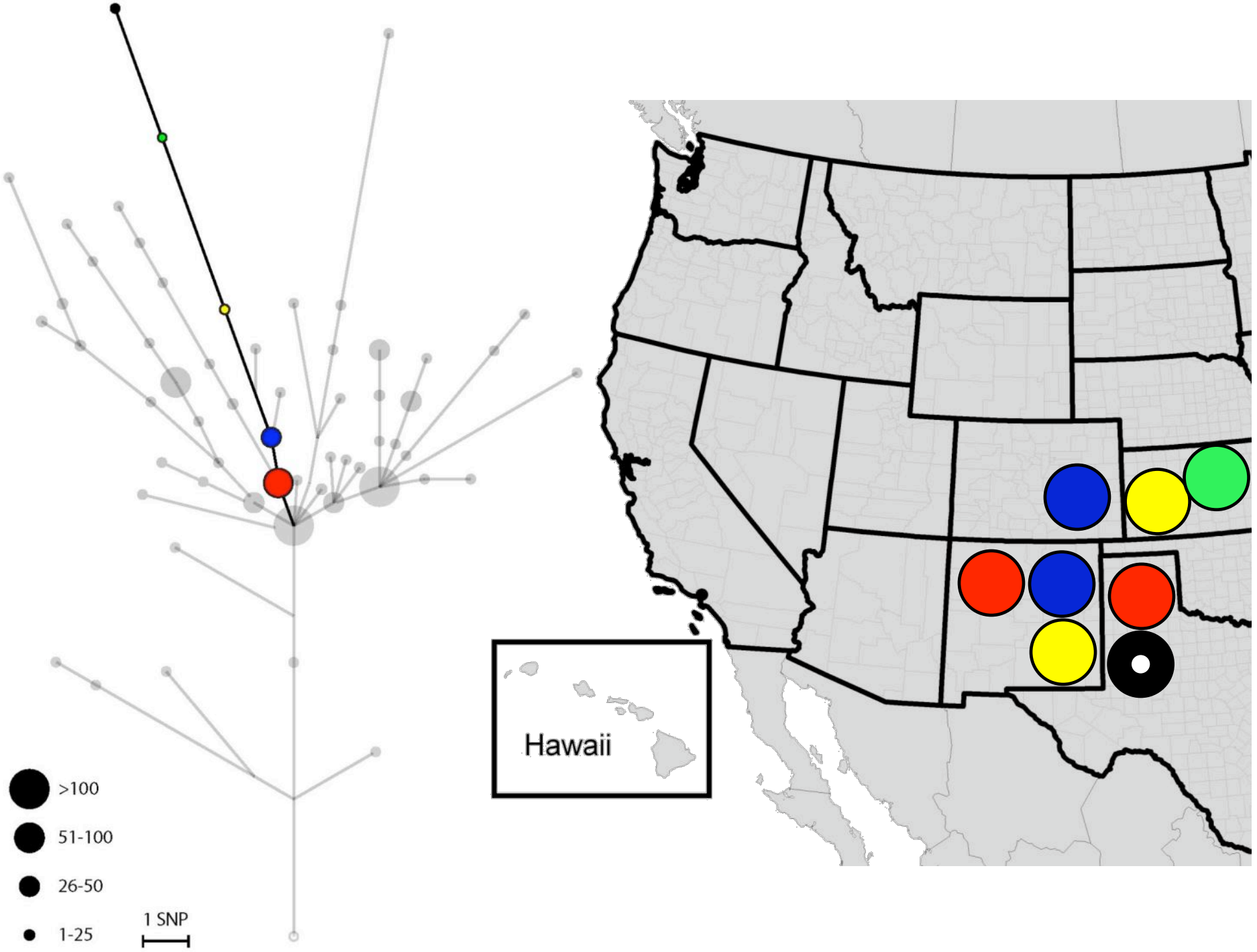

>100
51-100
26-50
1-25
1 SNP
Hawaii


>100
51-100
26-50
1-25
1 SNP
Hawaii


>100
51-100
26-50
1-25
1 SNP
Hawaii


>100
51-100
26-50
1-25
1 SNP
Hawaii


>100
51-100
26-50
1-25
1 SNP
Hawaii


>100
51-100
26-50
1-25
1 SNP
Hawaii

Conclusions: Continental USA

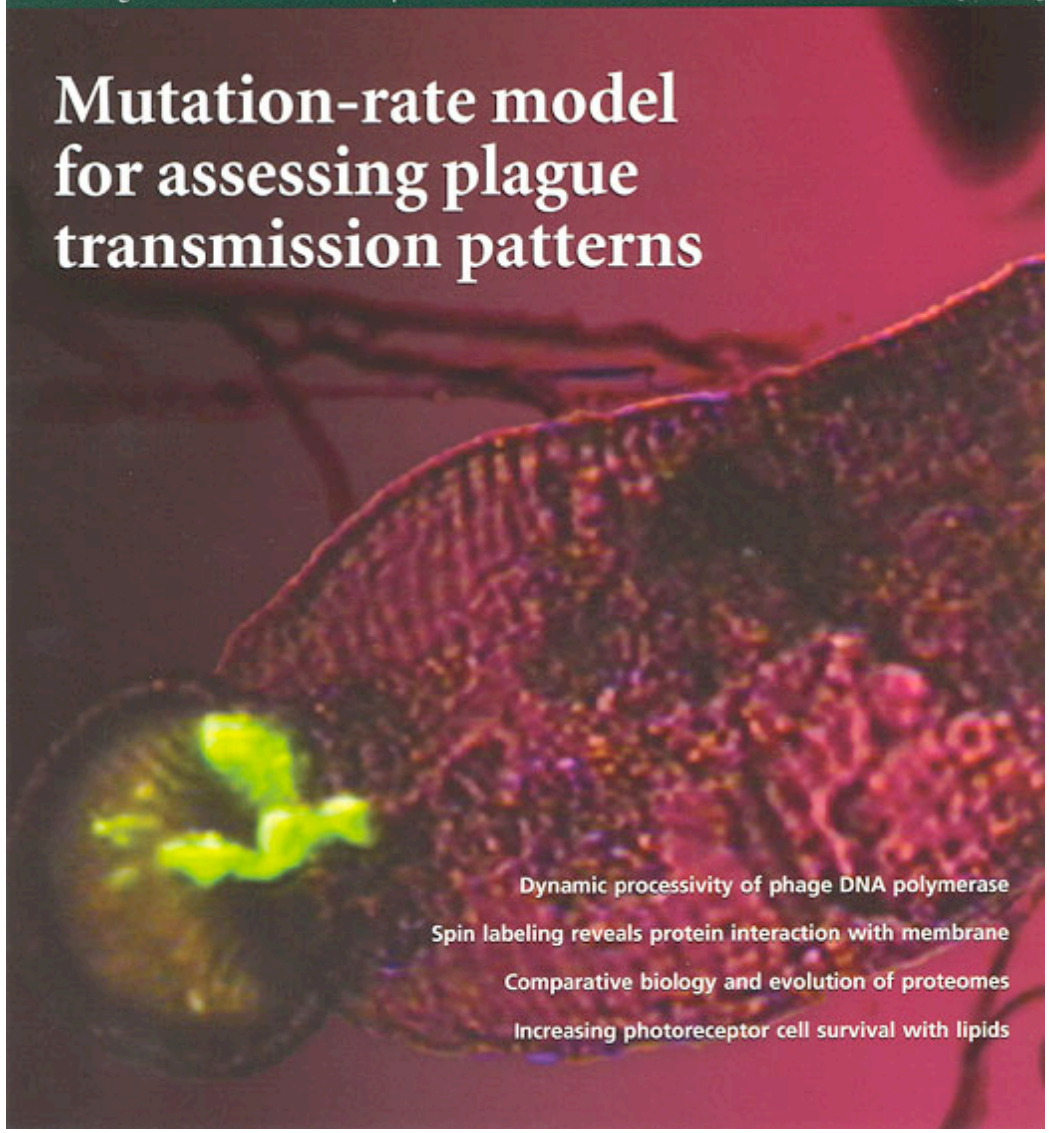
- Just one or maybe two introductions to native rodents
- No evidence for a selective sweep
  - Rather, star phylogeny originating from California
  - Suggests no adaptive benefits for any given lineage
- Evidence for rapid, complex dispersal
  - Many populations found in multiple states
  - Movement to the east but also back to the west
  - Representatives of diverse populations in same locality
- Question: How did plague spread so quickly in the US?

June 1, 2004 | vol. 101 | no. 22 | pp. 8255–8508

PNAS

Proceedings of the National Academy of Sciences of the United States of America www.pnas.org

Mutation-rate model for assessing plague transmission patterns

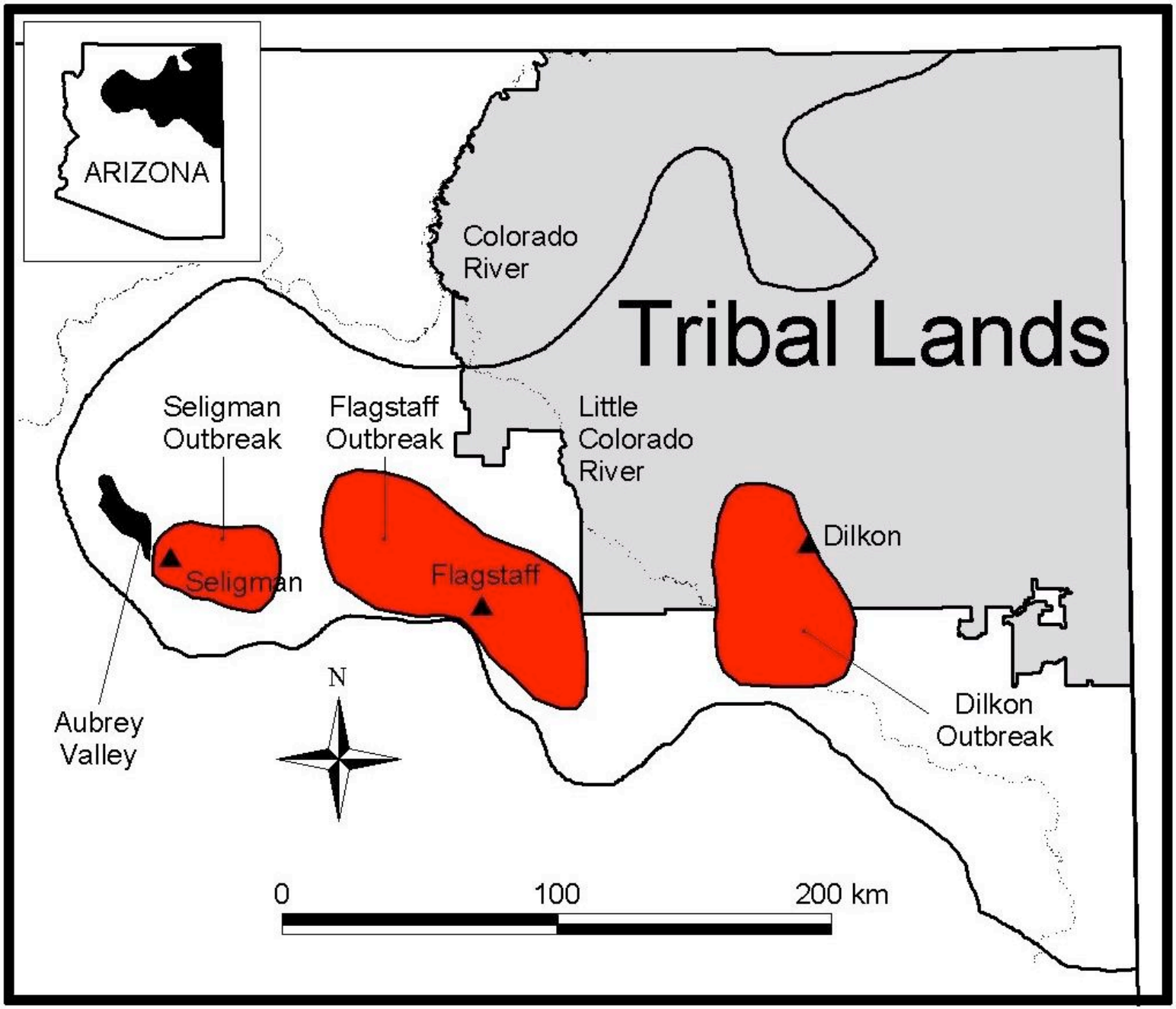


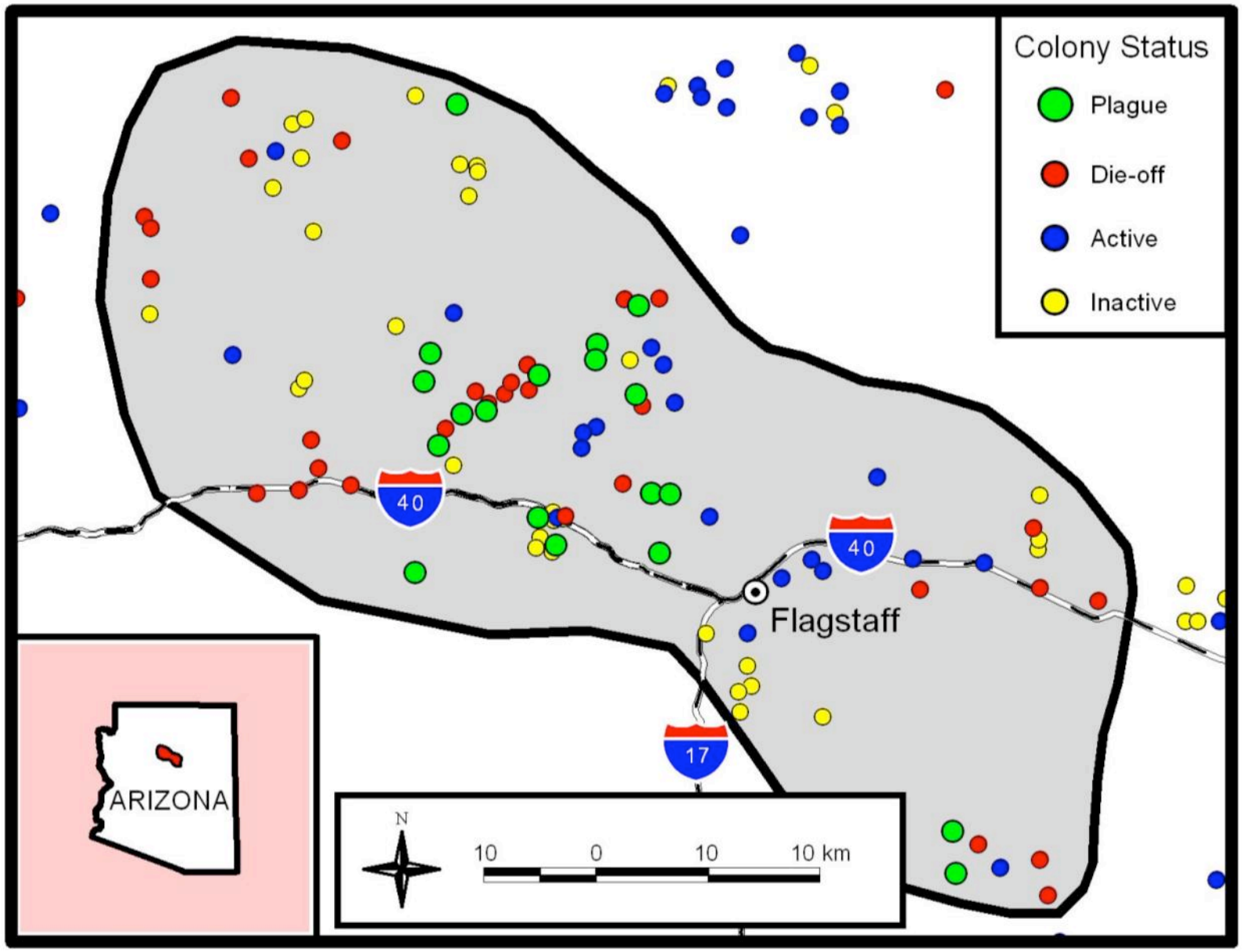
Dynamic processivity of phage DNA polymerase

Spin labeling reveals protein interaction with membrane

Comparative biology and evolution of proteomes

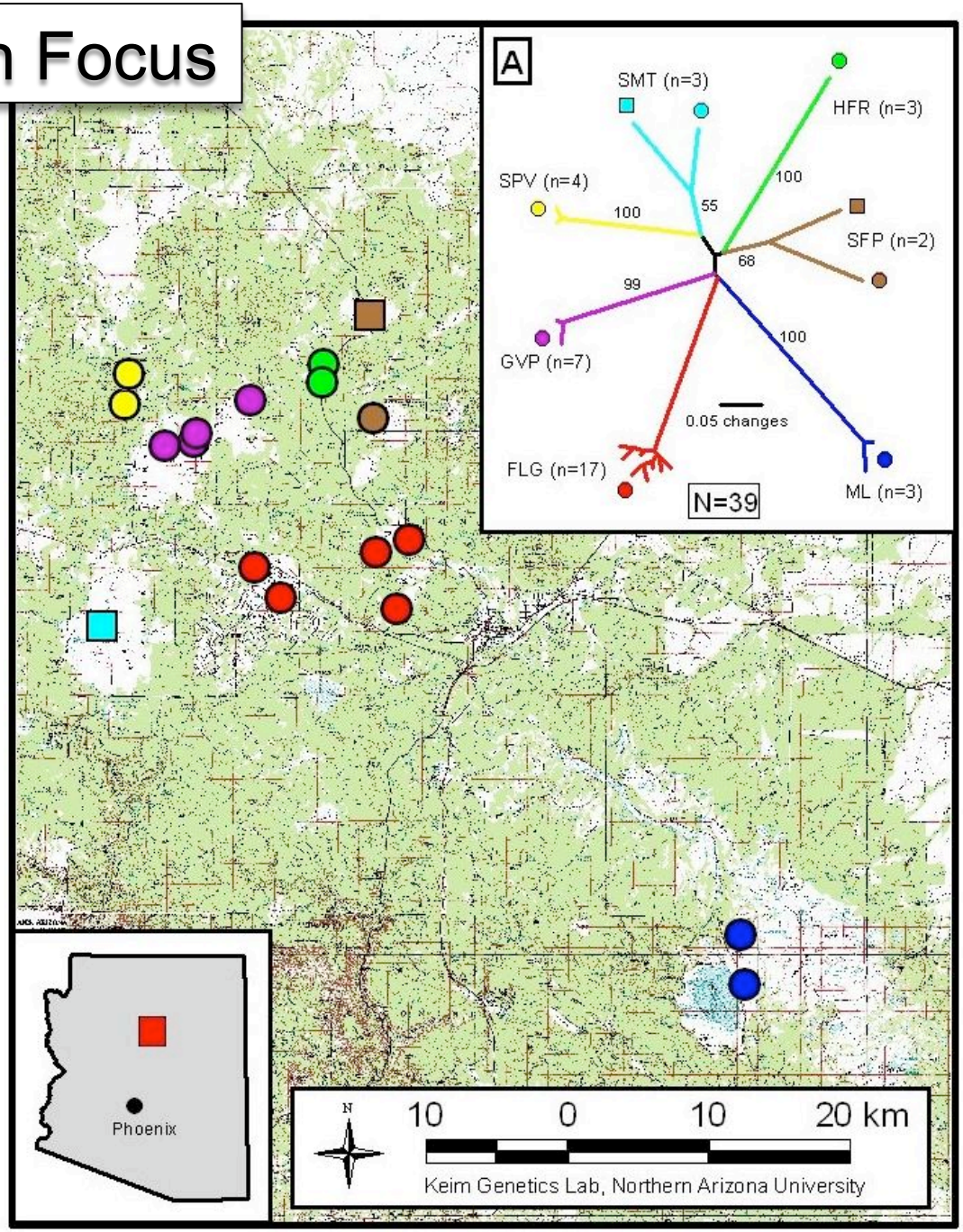
Increasing photoreceptor cell survival with lipids


ARIZONA
Colorado River
Tribal Lands
Seligman Outbreak
Flagstaff Outbreak
Little Colorado River
Dilkon
Seligman
Flagstaff
Aubrey Valley
N
Dilkon Outbreak
0
100
200 km



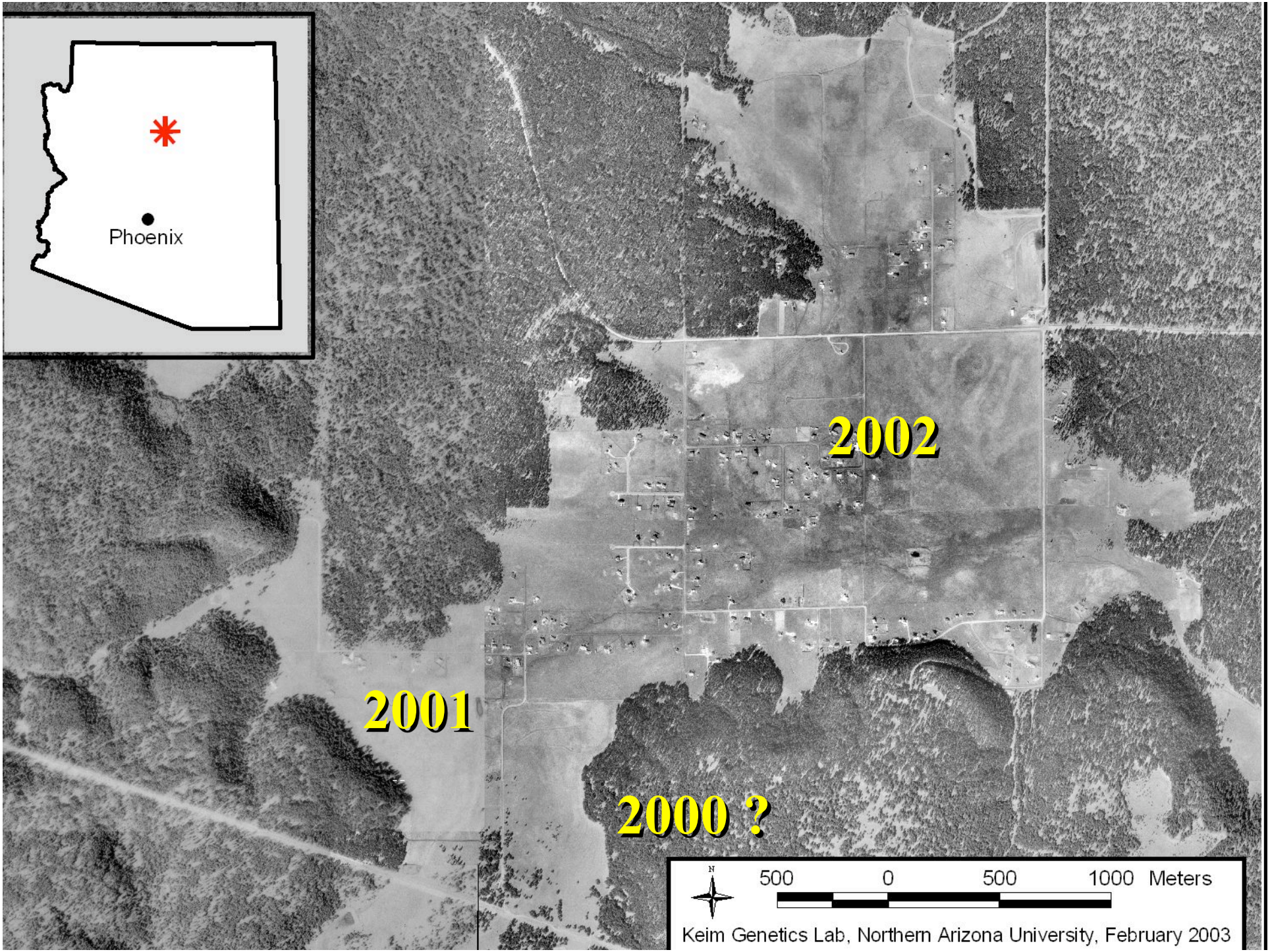
Wagner *et al*. 2004. PNAS.

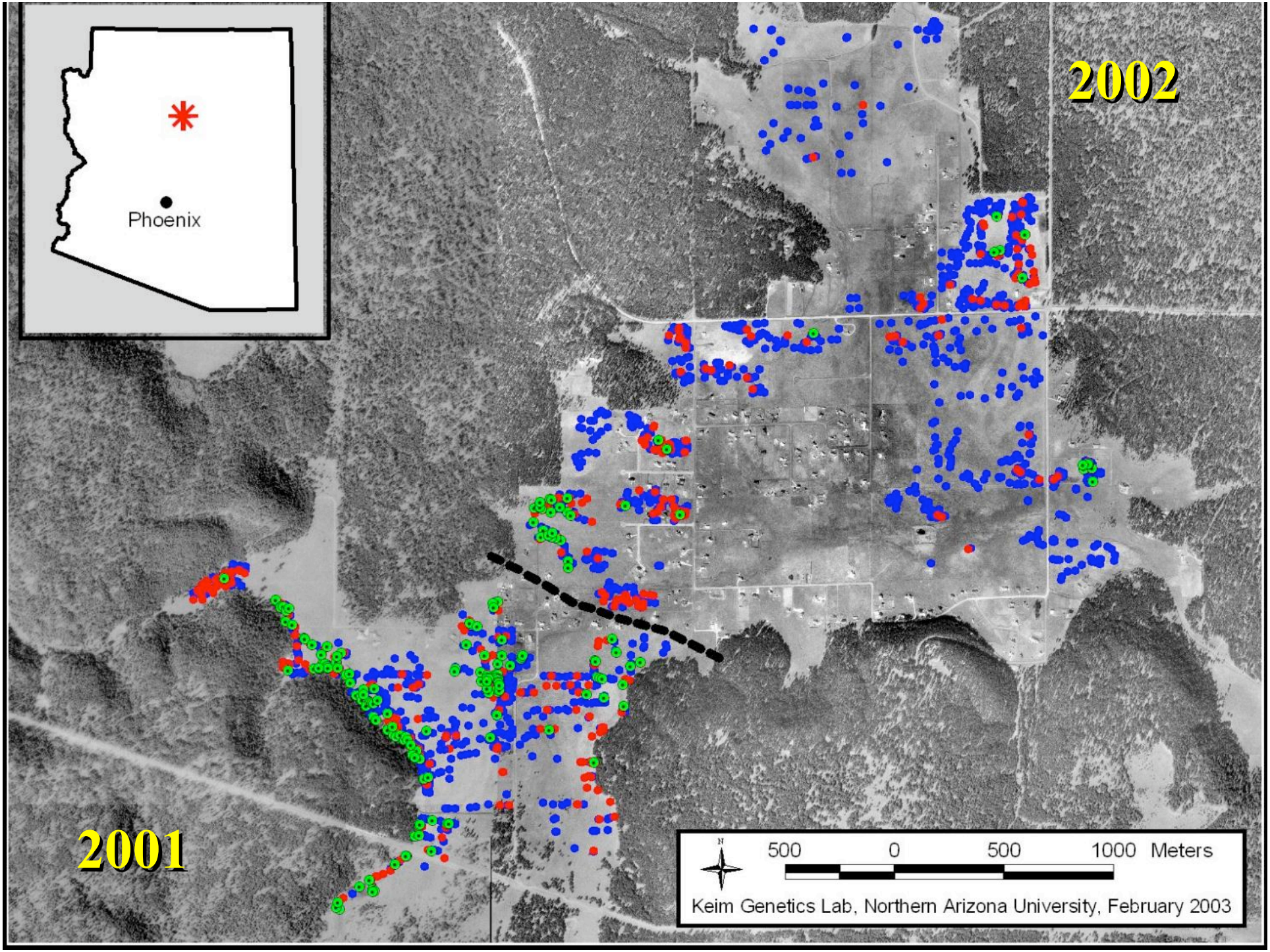
50+ Season Focus



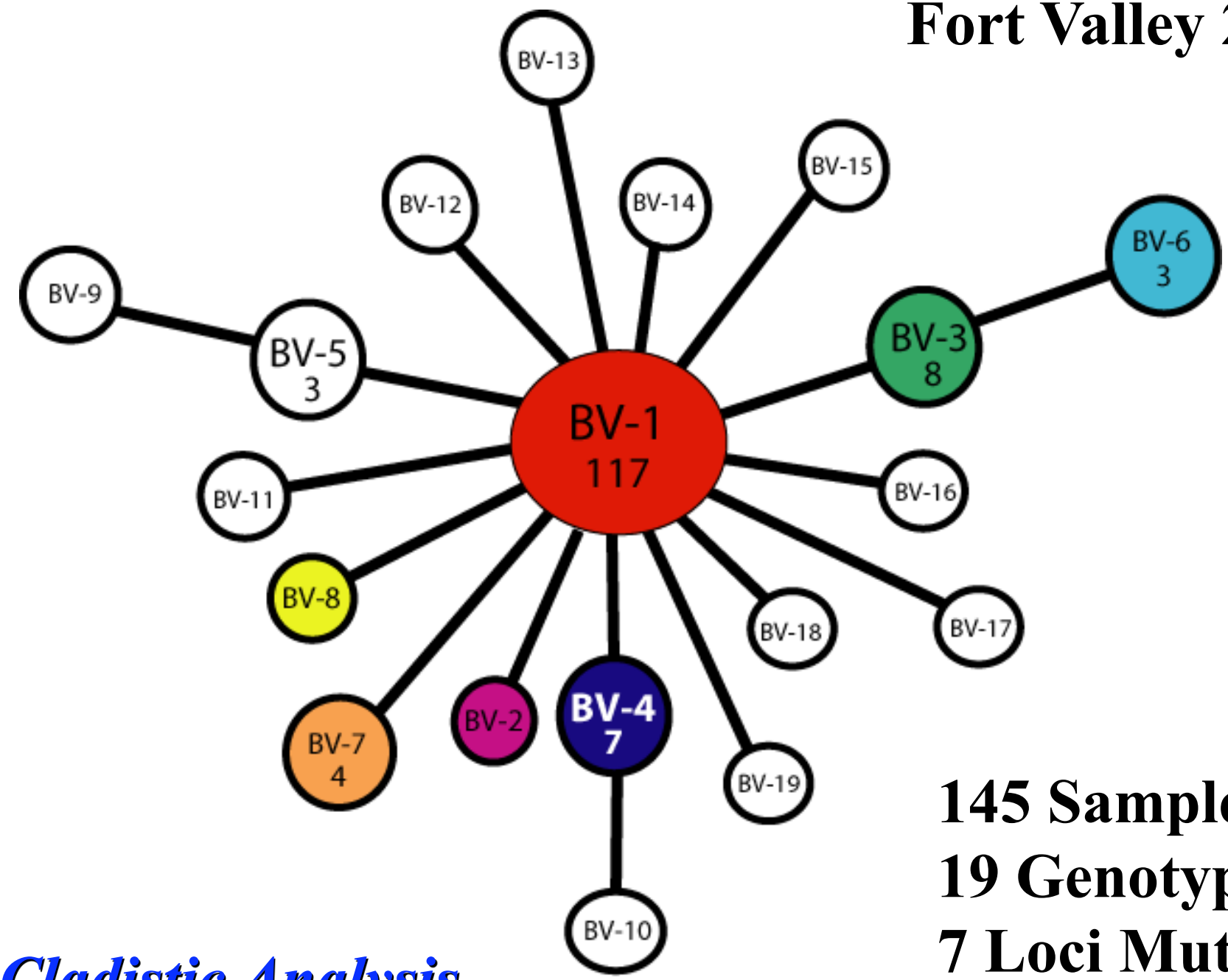
Girard and Wagner et al. 2004.

PNAS **101**:8409-8413.


Phoenix
2002
2001
2000 ?
N
500
0
500
1000 Meters
Keim Genetics Lab, Northern Arizona University, February 2003


Phoenix
2002
2001
500
0
500
1000 Meters
Keim Genetics Lab, Northern Arizona University, February 2003

Fort Valley 2001



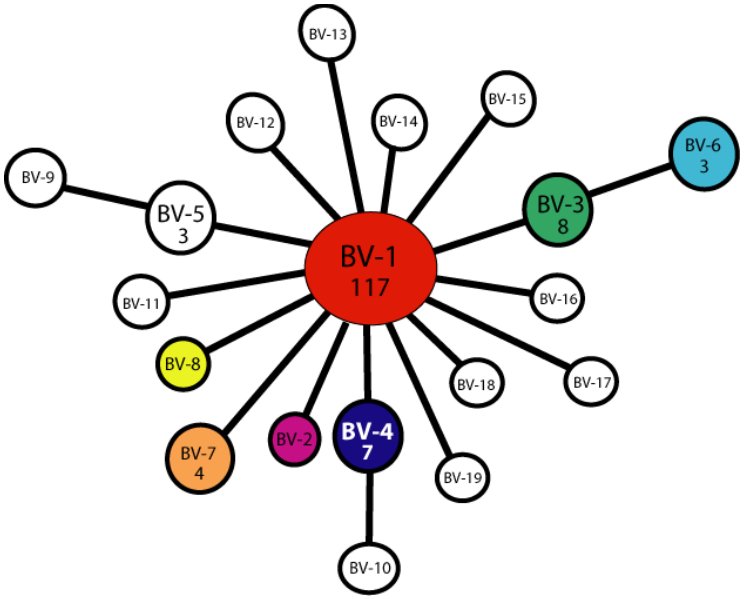
145 Samples
19 Genotypes
7 Loci Mutated

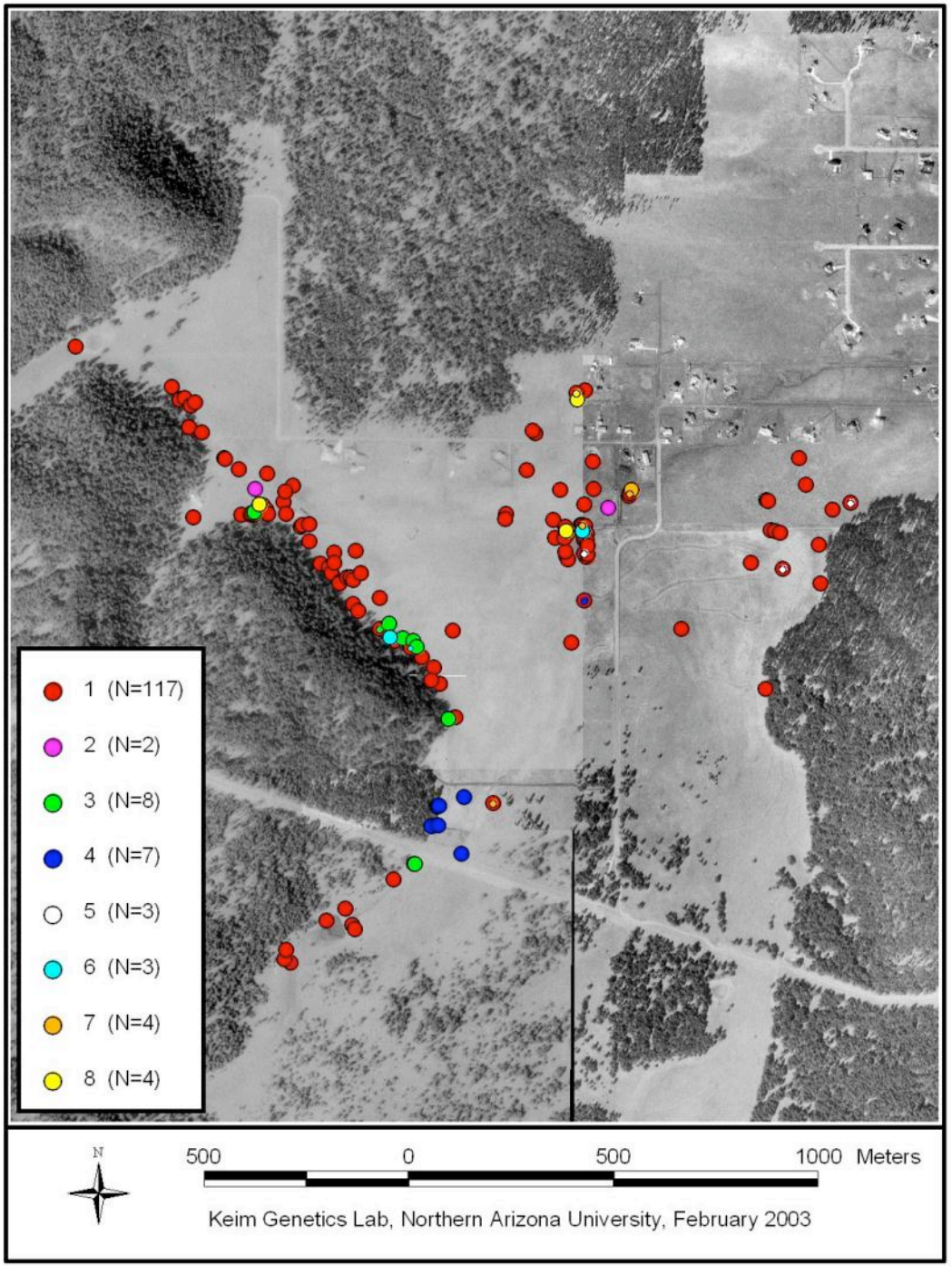
Cladistic Analysis

Plague Genotype Distribution

Fort Valley, AZ

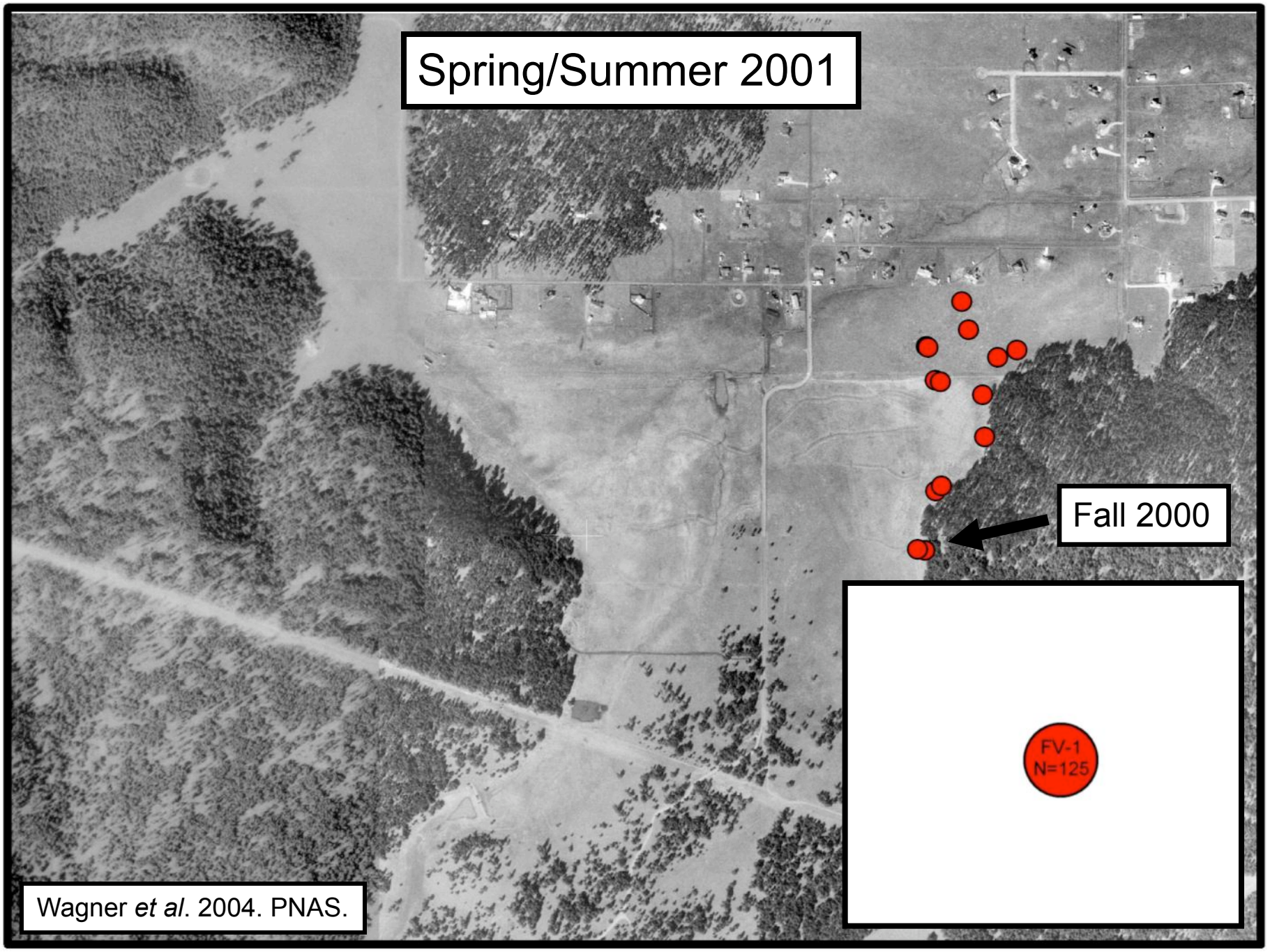
2001

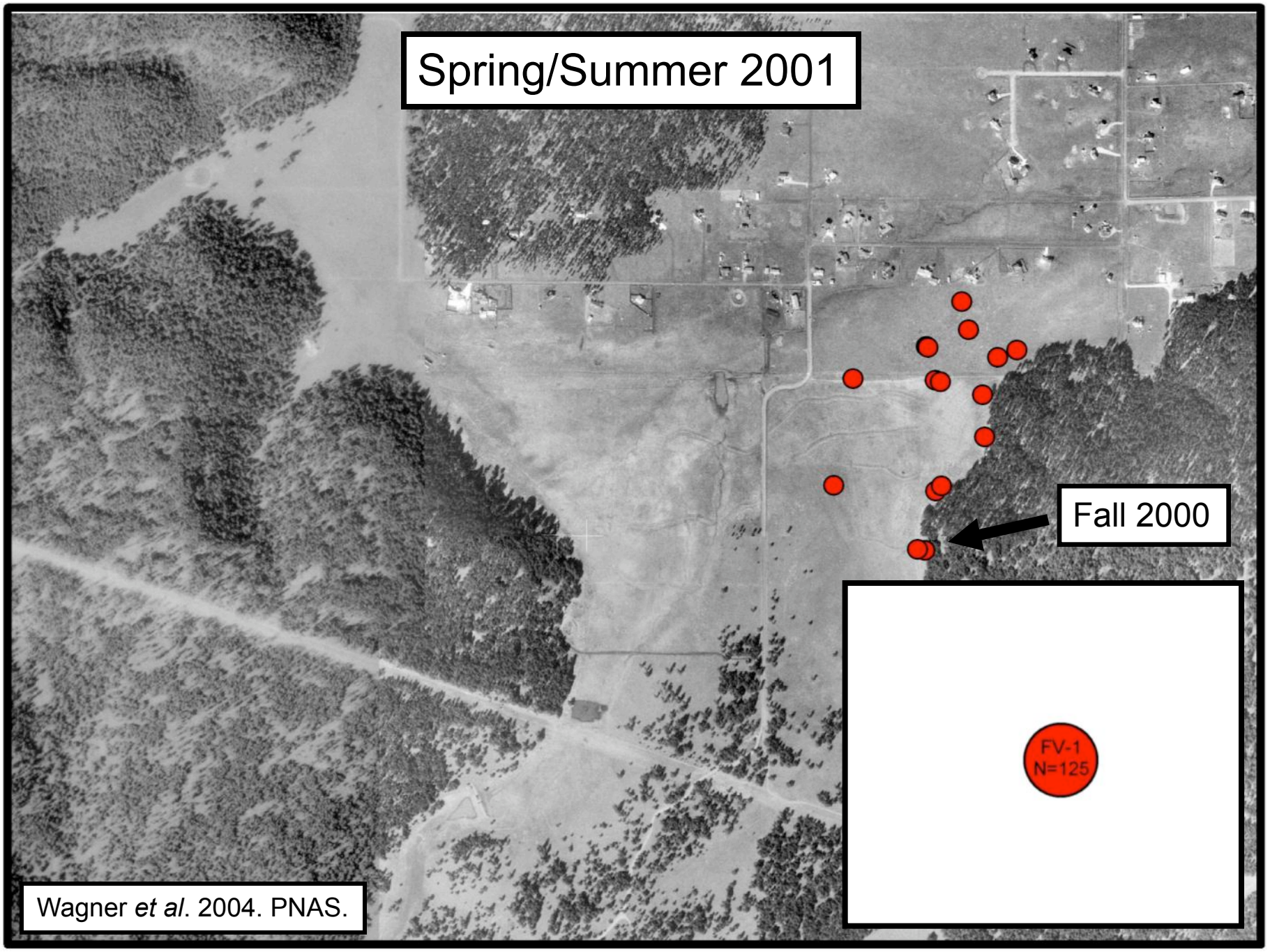


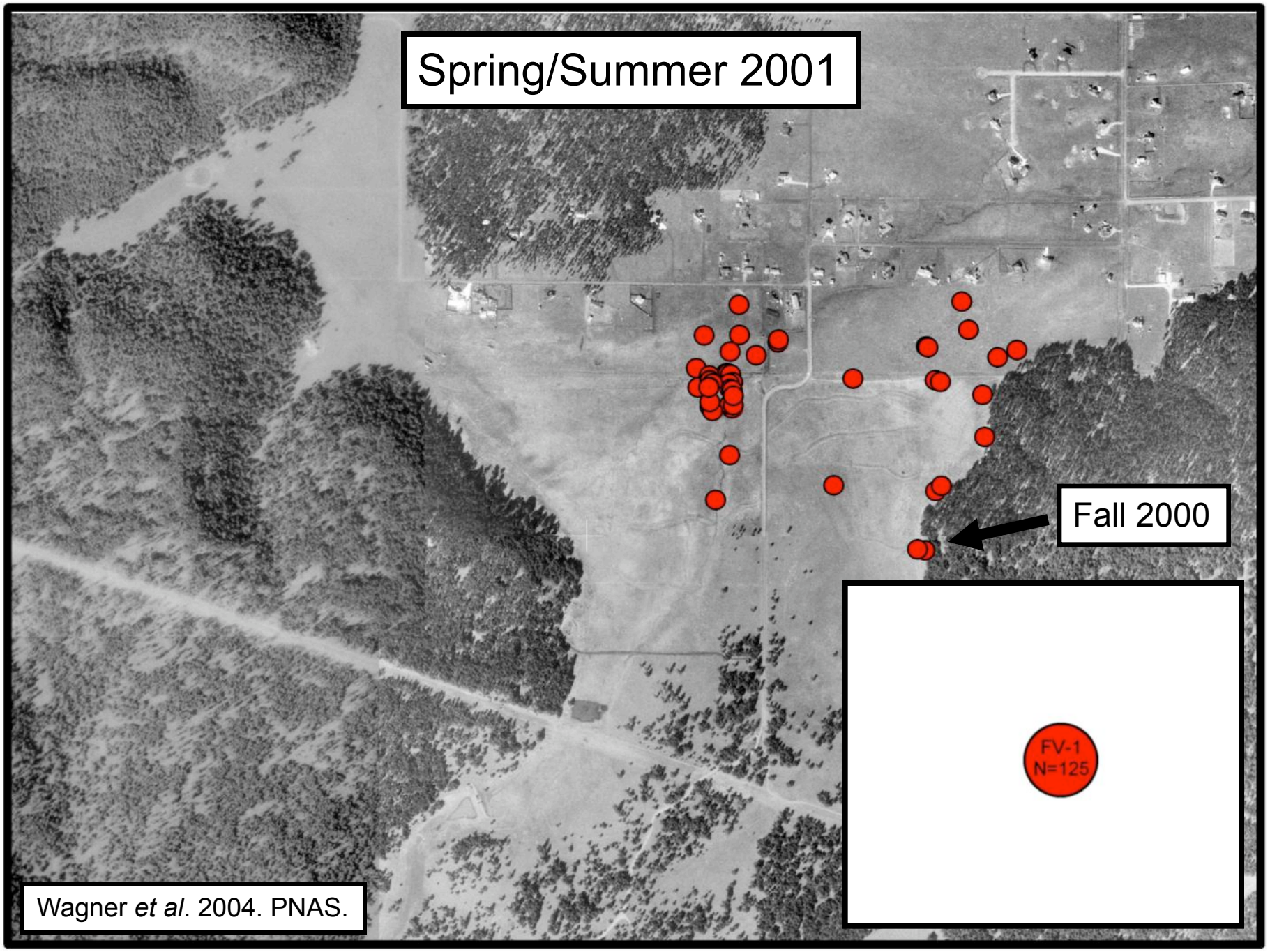


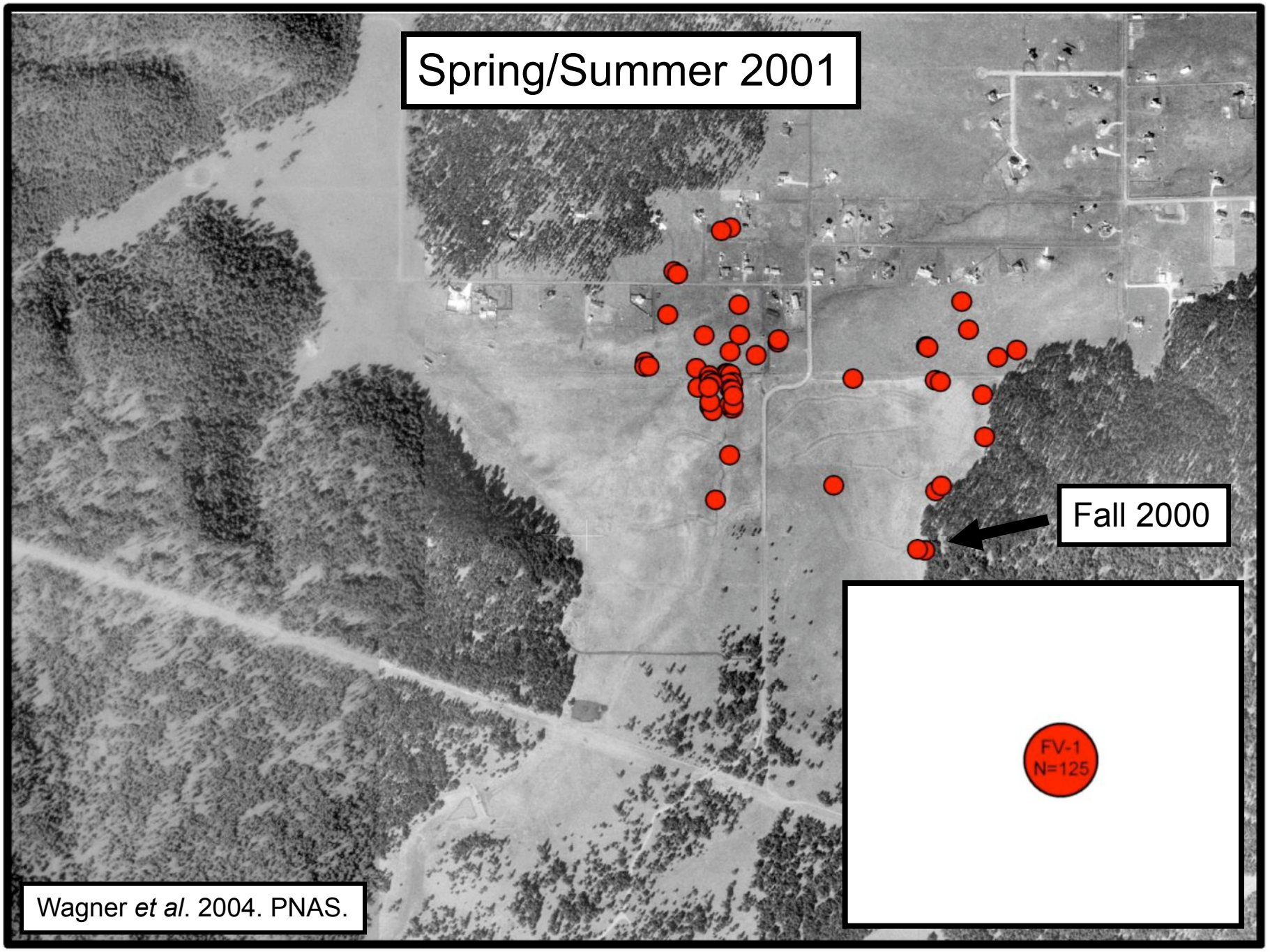

Wagner *et al*. 2004. PNAS.

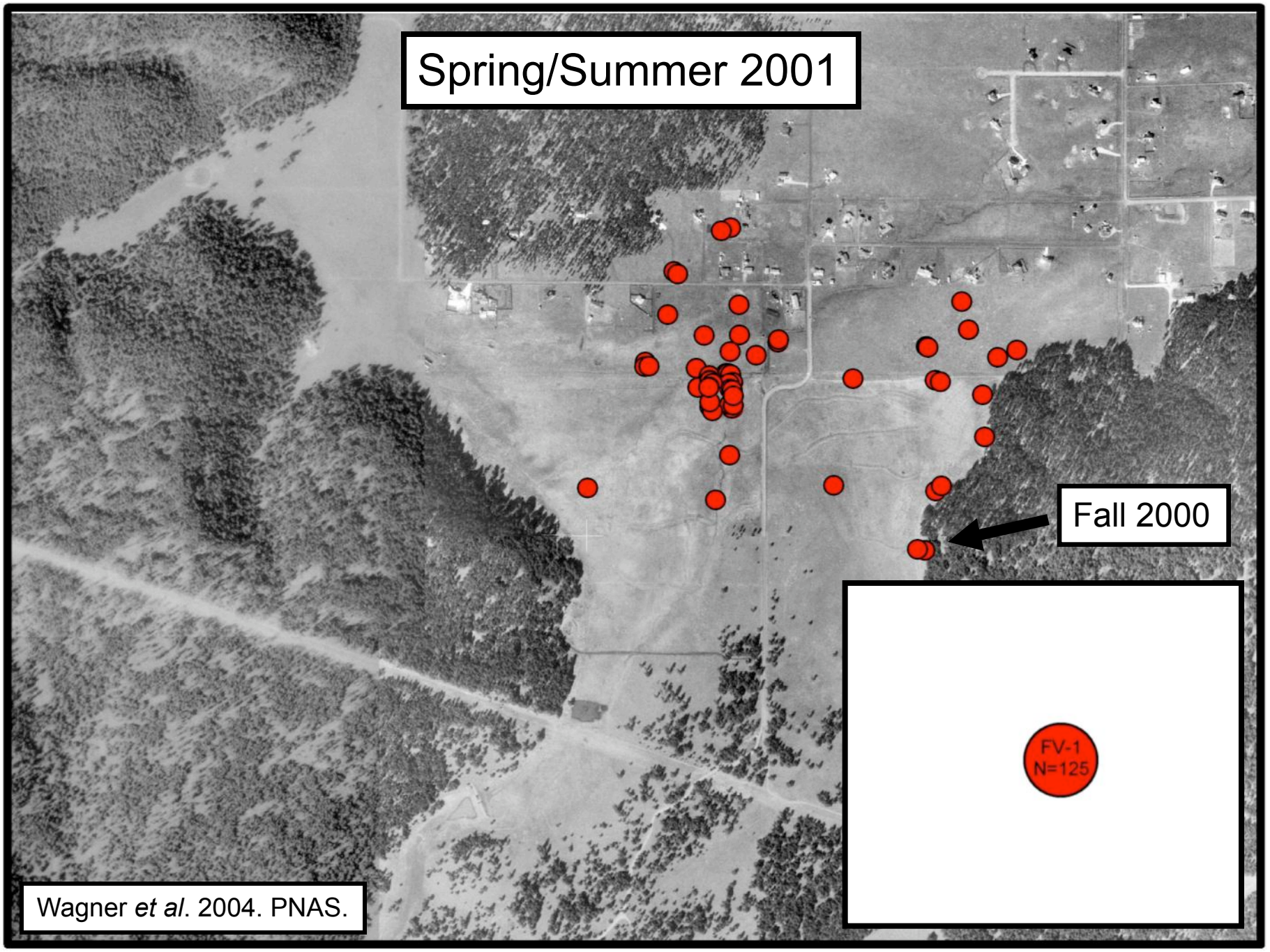

Fall 2000
FV-1
N=125
Wagner *et al*. 2004. PNAS.

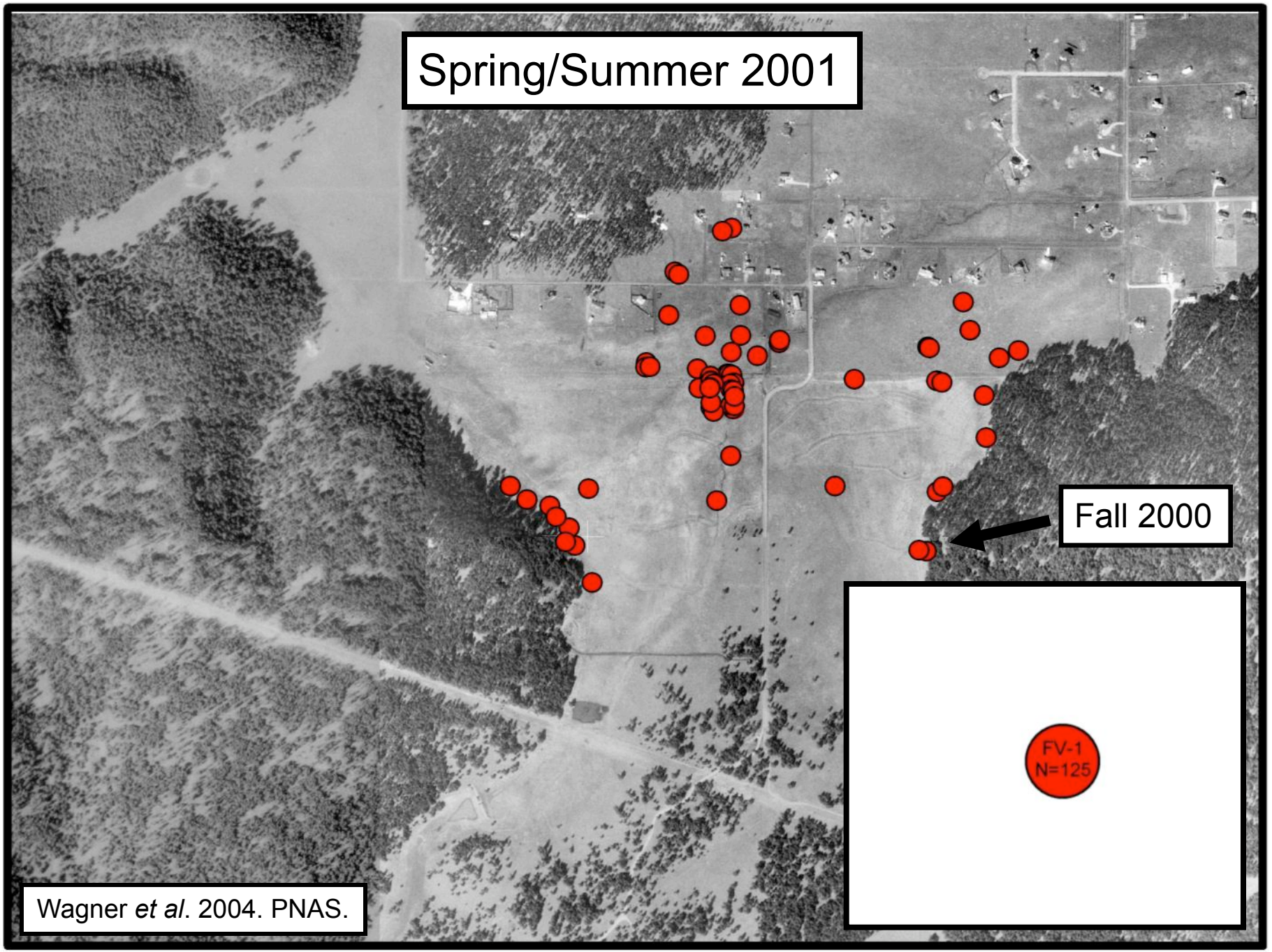

Spring/Summer 2001
Fall 2000
FV-1
N=125
Wagner *et al*. 2004. PNAS.

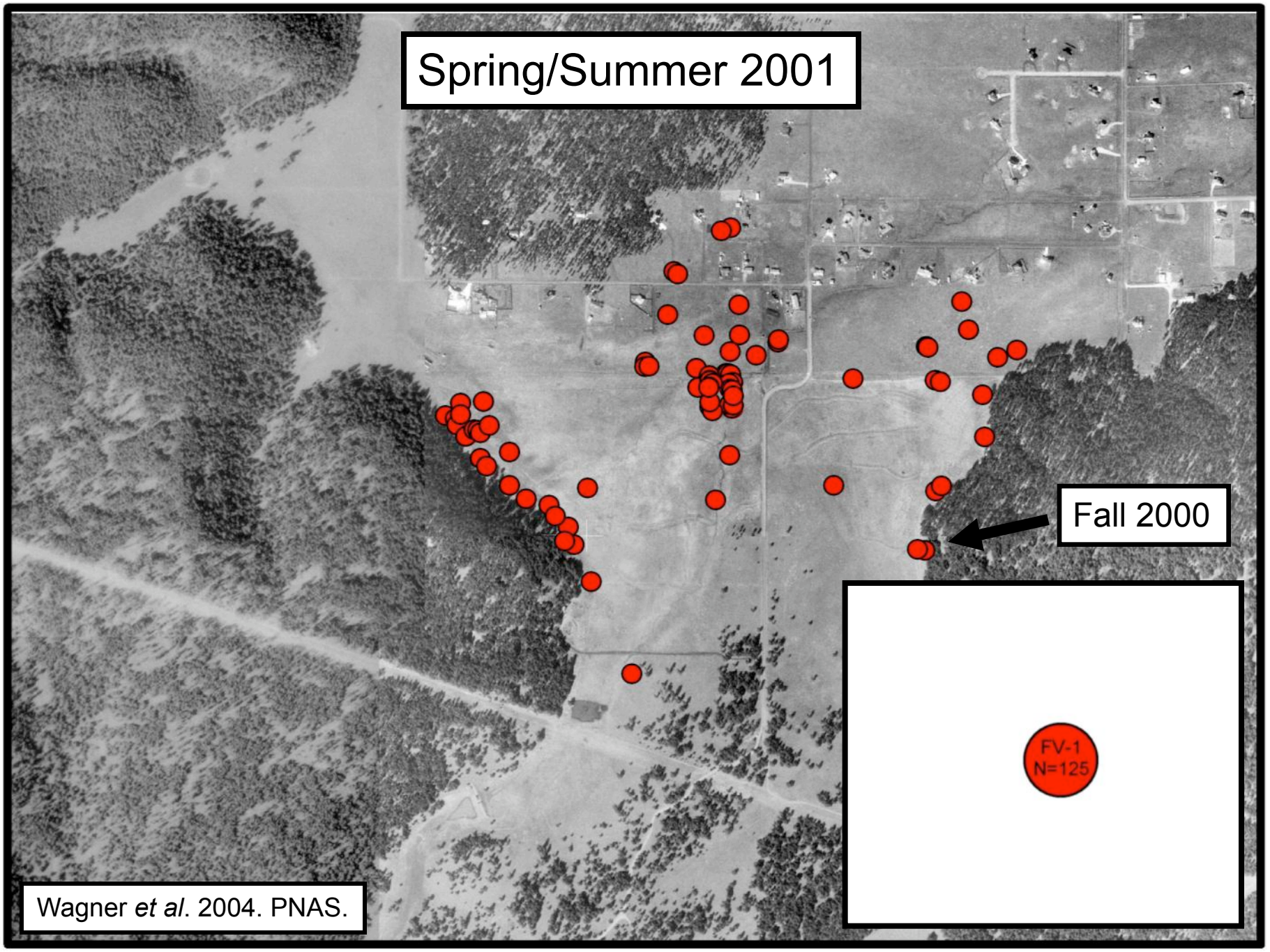

Spring/Summer 2001
Fall 2000
FV-1
N=125
Wagner *et al*. 2004. PNAS.

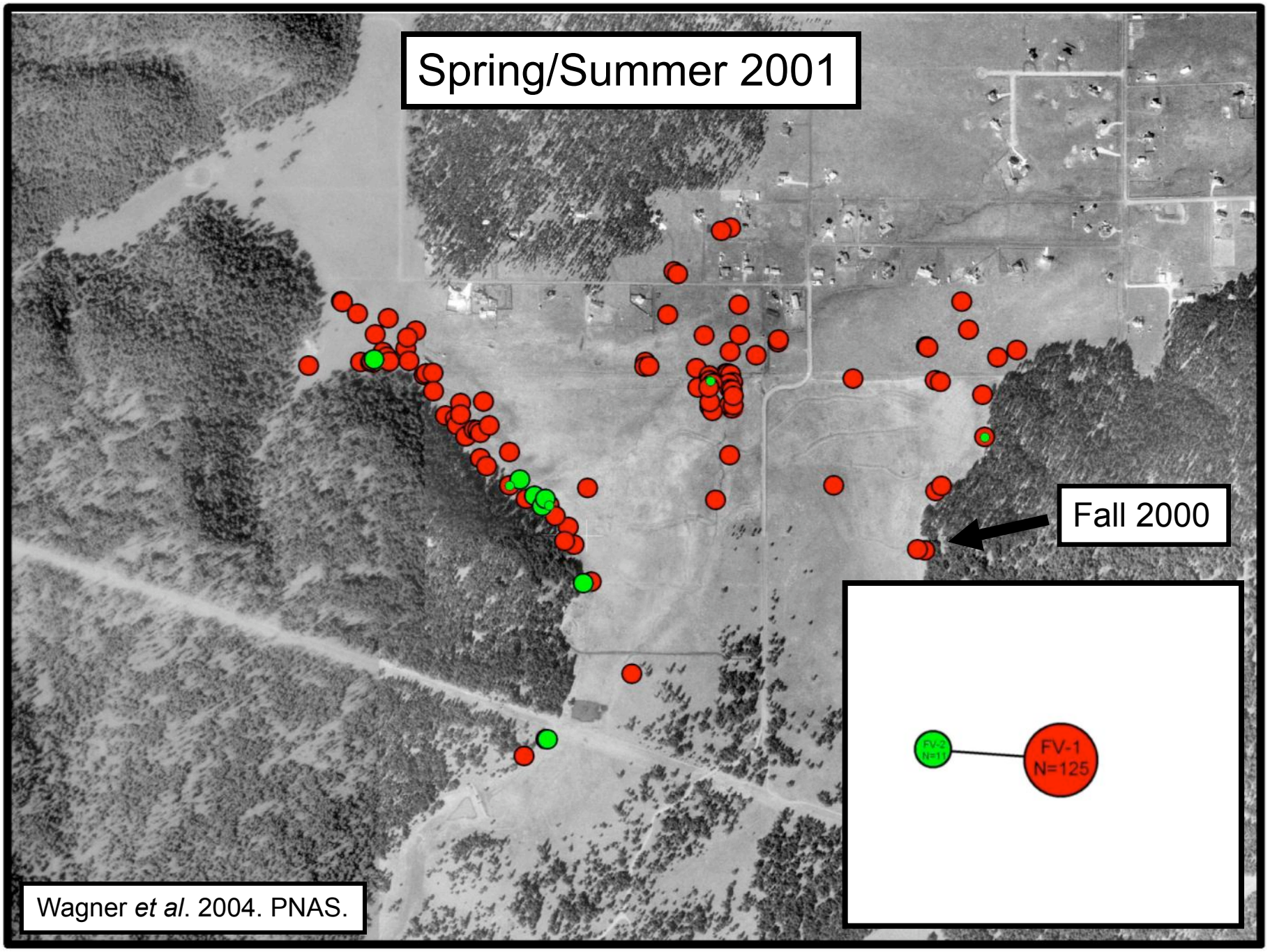

Spring/Summer 2001
Fall 2000
FV-1
N=125
Wagner *et al*. 2004. PNAS.

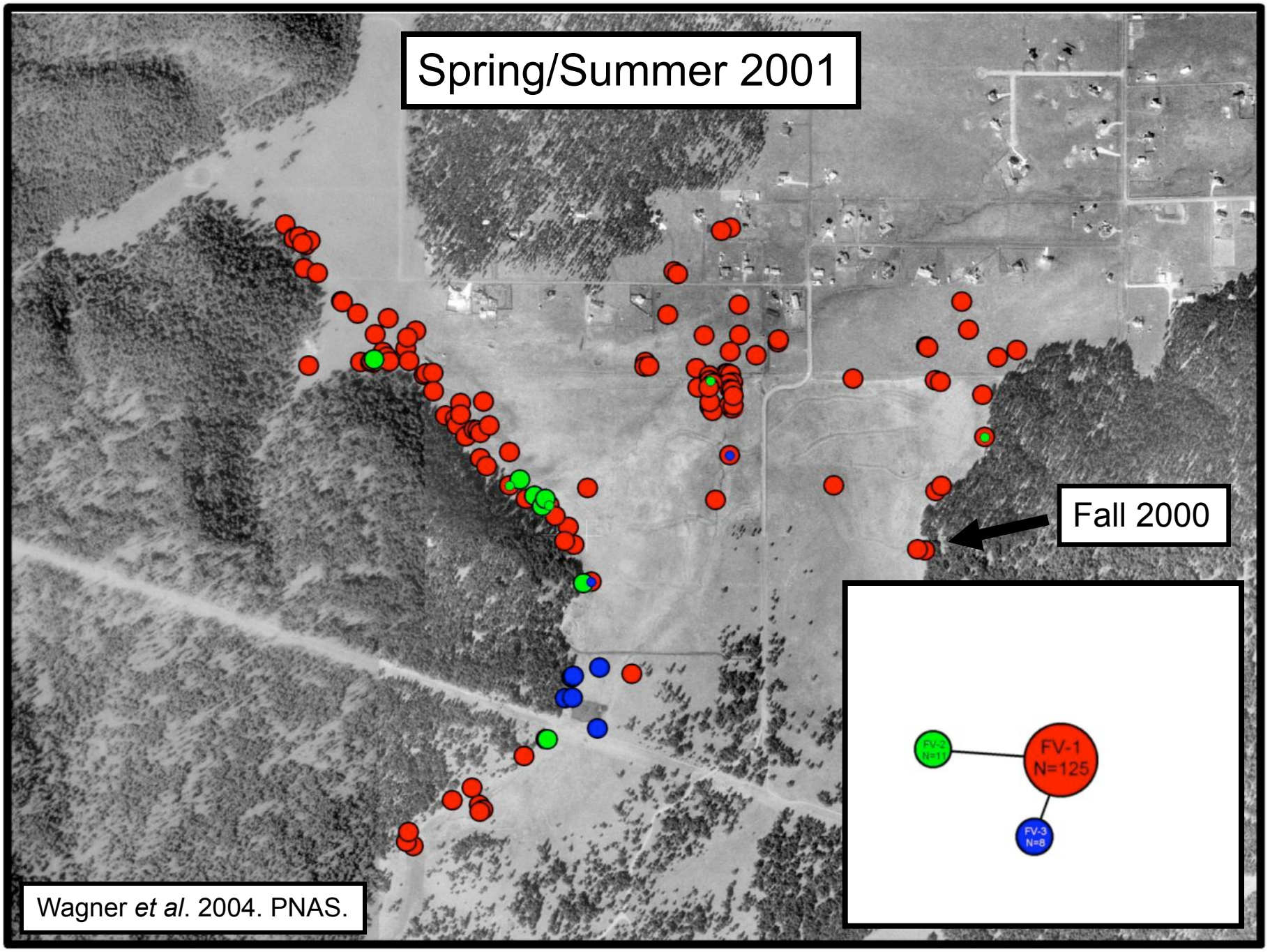

Spring/Summer 2001
Fall 2000
FV-1
N=125
Wagner *et al*. 2004. PNAS.

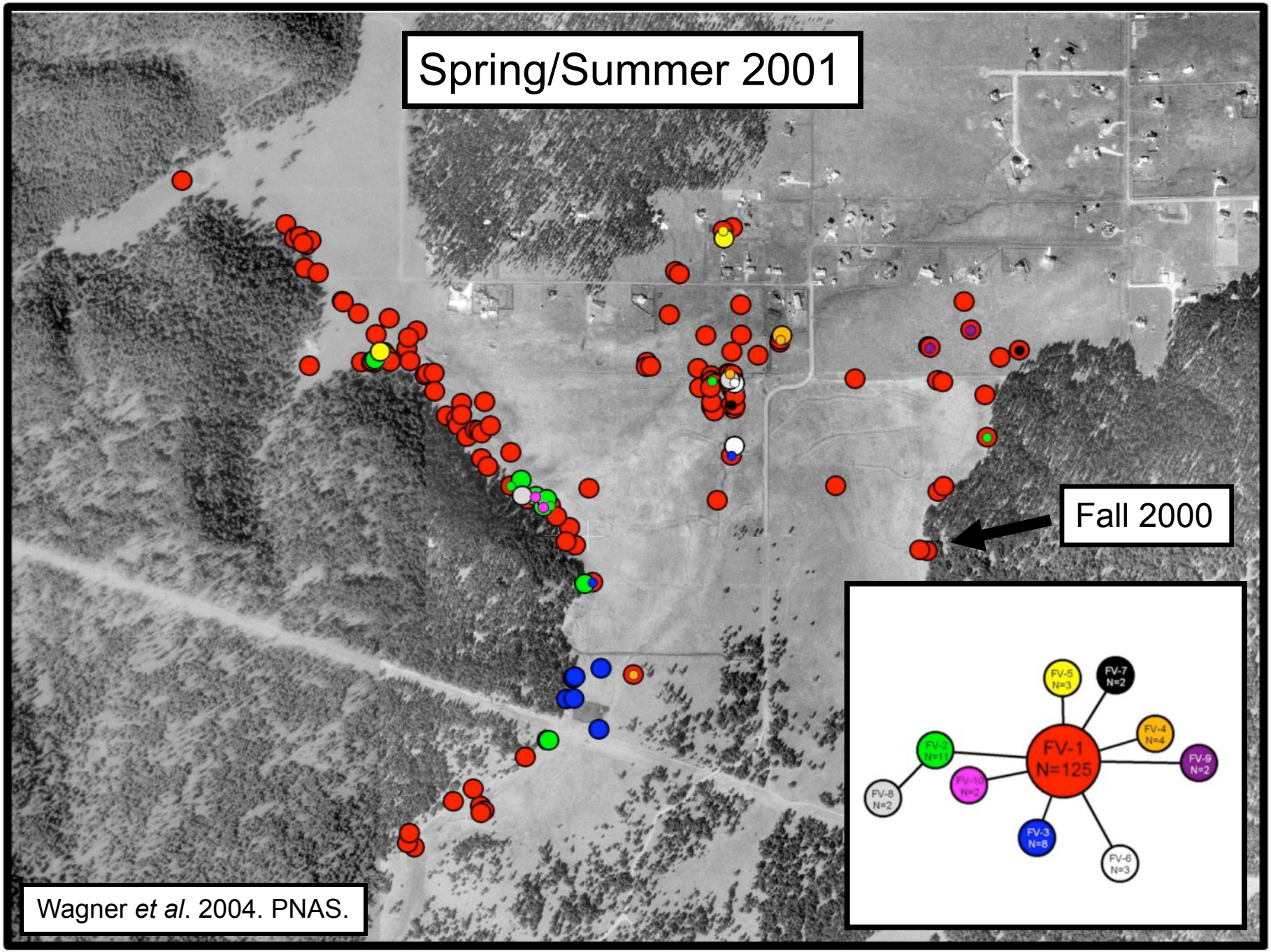

Spring/Summer 2001
Fall 2000
FV-1
N=125
Wagner *et al*. 2004. PNAS.


Spring/Summer 2001
Fall 2000
FV-1
N=125
Wagner *et al*. 2004. PNAS.

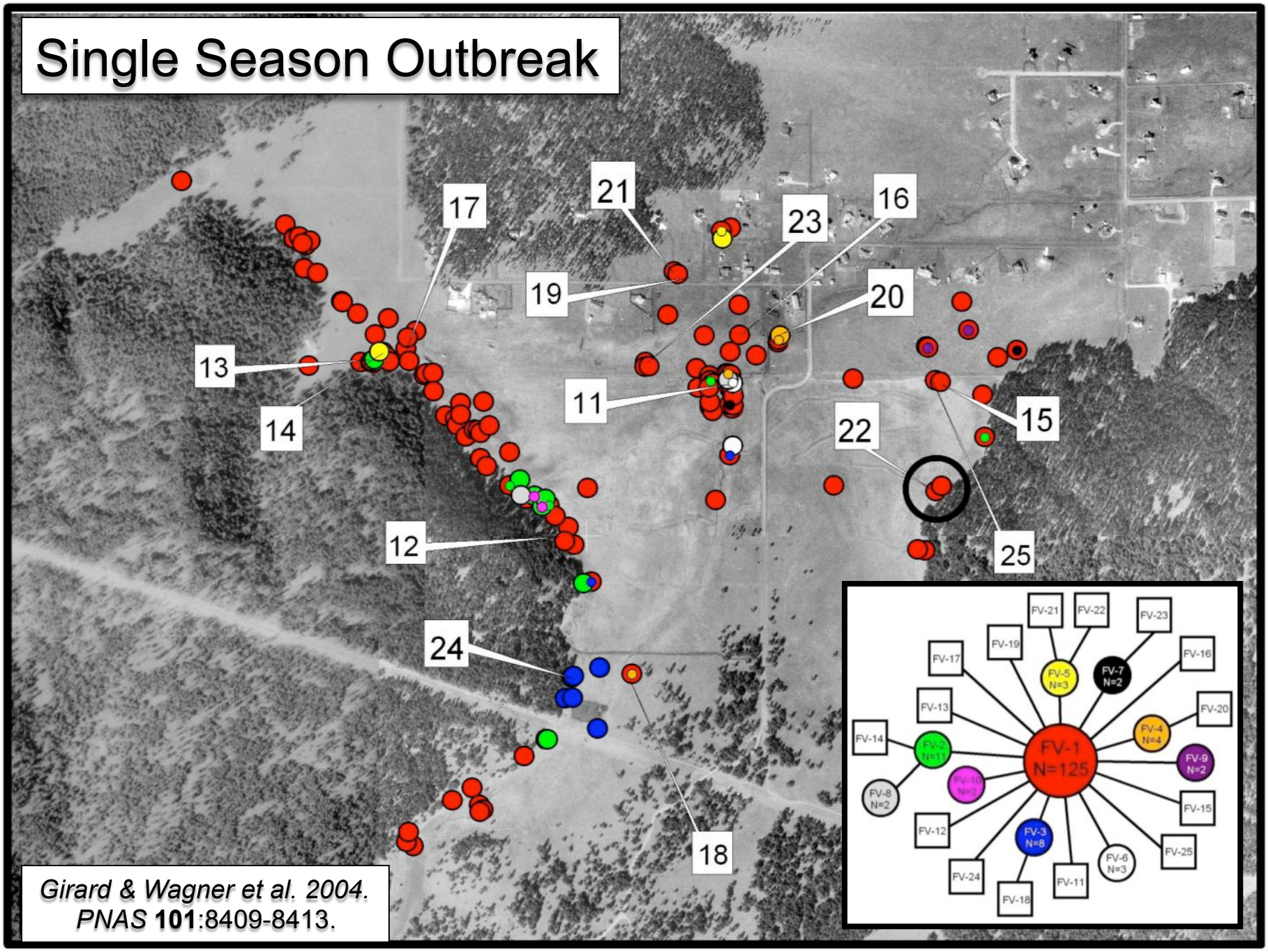

Spring/Summer 2001
Fall 2000
FV-1
N=125
Wagner *et al*. 2004. PNAS.


Spring/Summer 2001
Fall 2000
FV-2
N=1
FV-1
N=125
Wagner *et al*. 2004. PNAS.


Spring/Summer 2001
Fall 2000
FV-2
N=1
FV-1
N=125
FV-3
N=8
Wagner *et al*. 2004. PNAS.


Spring/Summer 2001
Fall 2000
FV-1
N=125
FV-2
N=1
FV-3
N=8
FV-4
N=4
FV-5
N=3
FV-6
N=3
FV-7
N=2
FV-8
N=2
FV-9
N=2
FV-10
N=2
Wagner *et al*. 2004. PNAS.

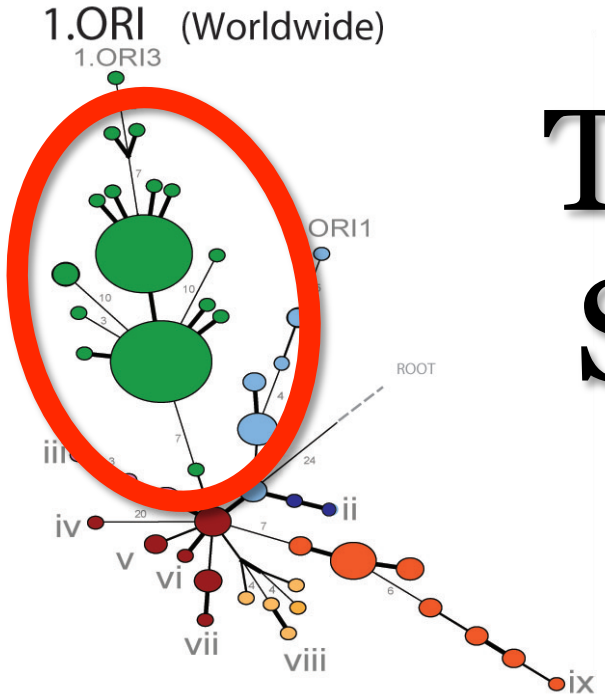
Single Season Outbreak

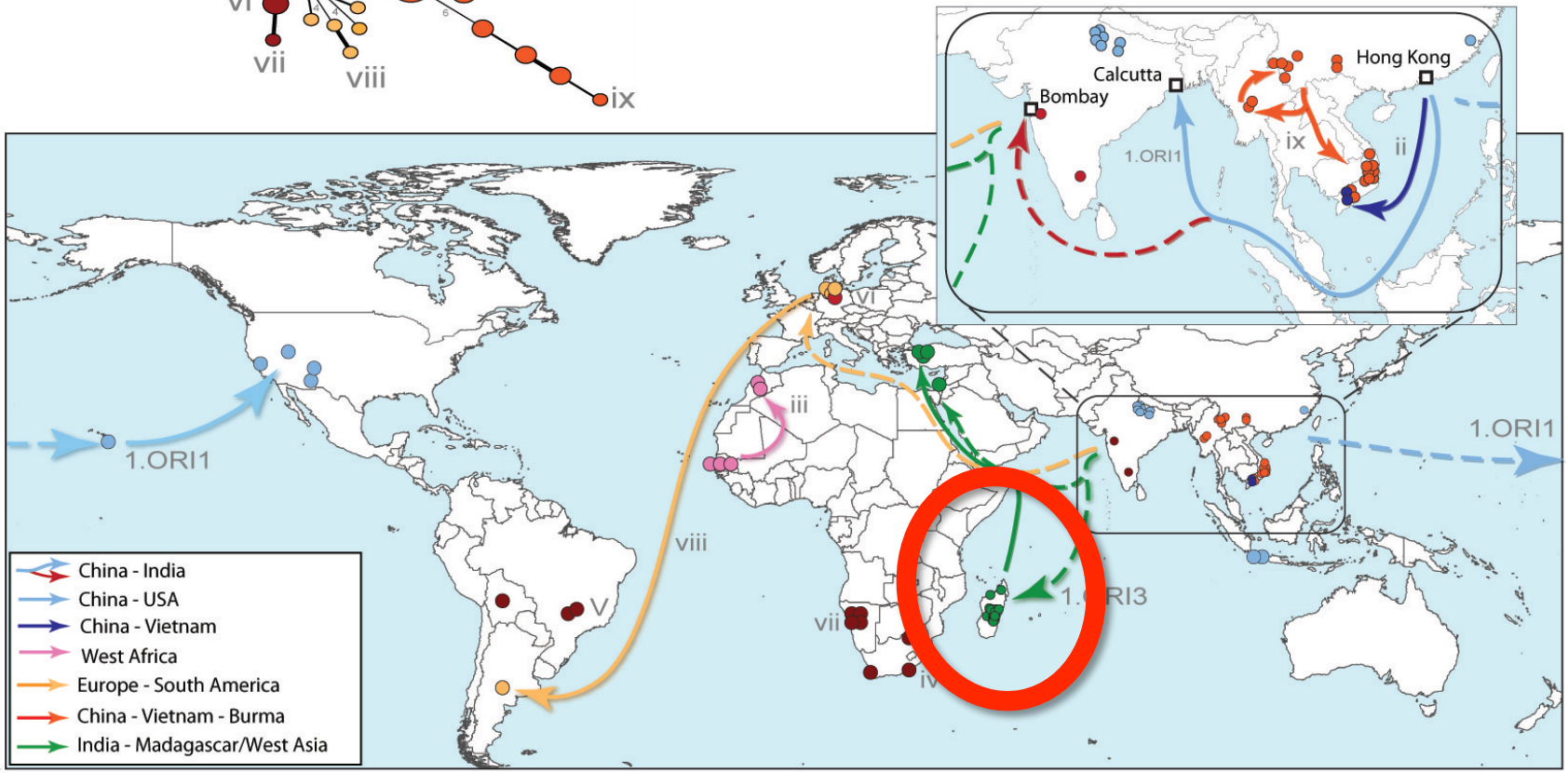


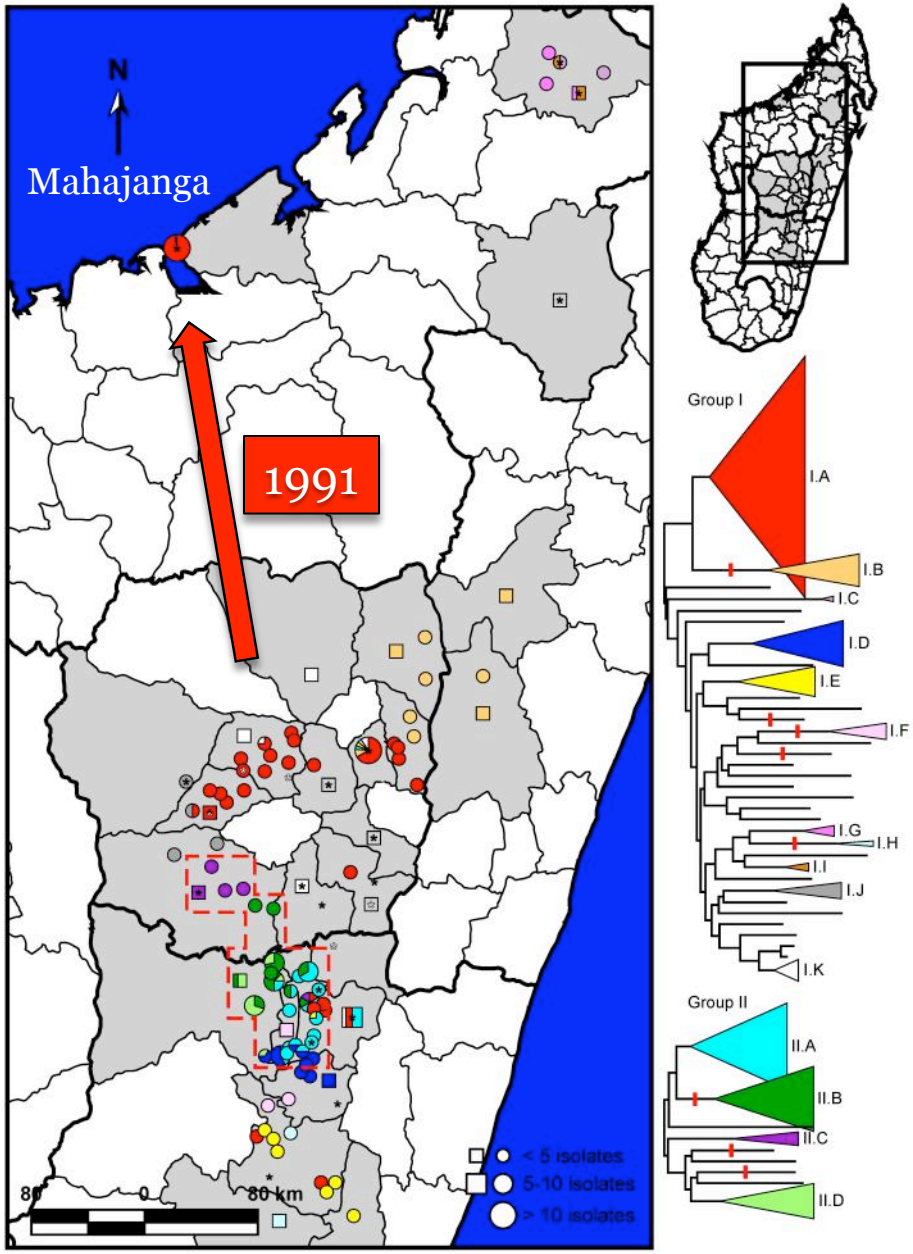
Girard & Wagner et al. 2004.
PNAS **101**:8409-8413.

Third Pandemic: Spread of 1.ORI

Morelli *et al.* 2010. Nature Genetics.





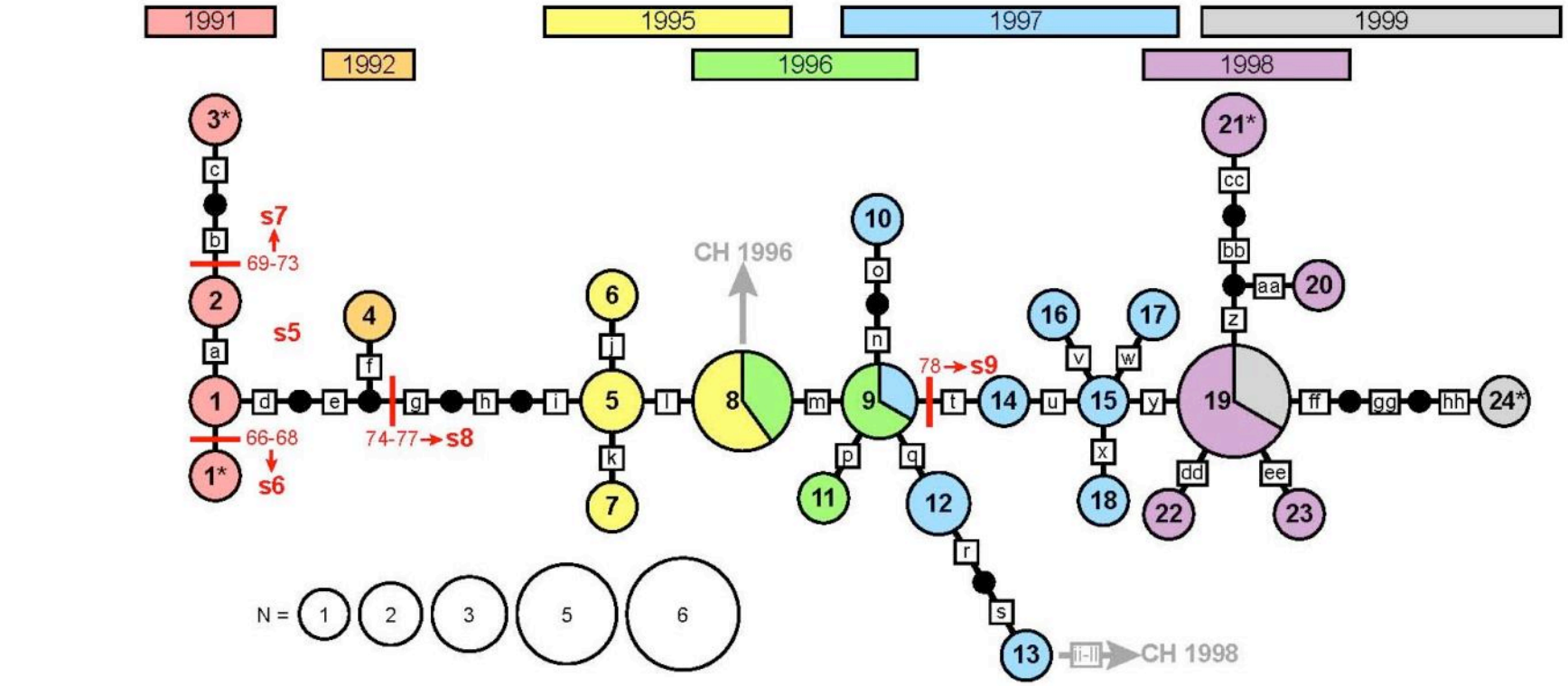


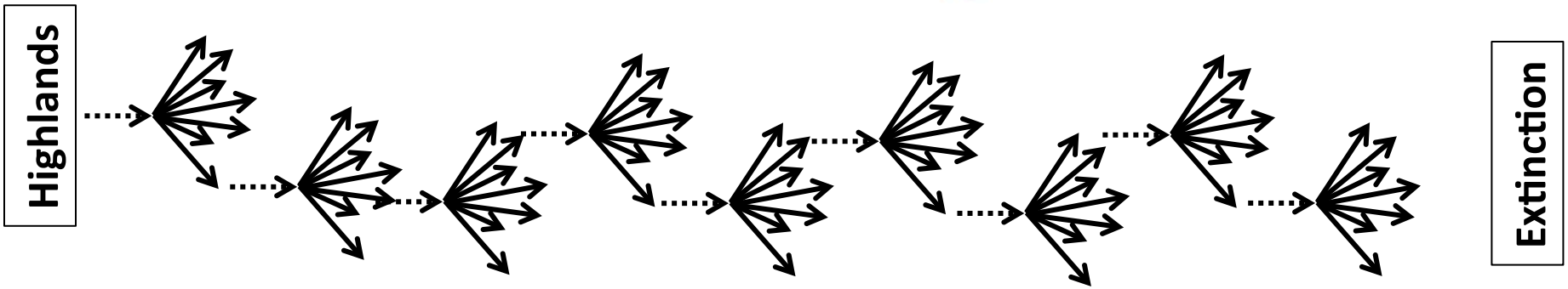
Madagascar

- Two major groups
- 201 unique genotypes out of 221 isolates
- 11 major clusters
- Mantel Regression r=0.006 (p=0.0002)

Vogler et al. 2011. *PLoS Neg. Trop. Dis.* 5(9): e1319.

Mahajanga Plague Phylogeny





Vogler, unpublished data

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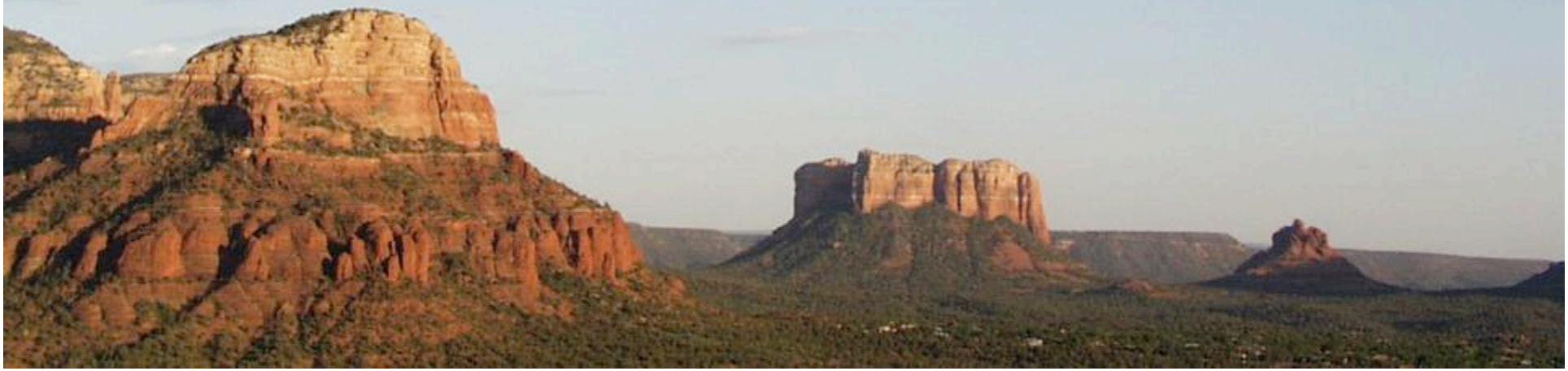
Investigating a laboratory acquired case of plague.

Paul Keim, PhD

Northern Arizona University

The Translational Genomics Research Institute



Case History:
A University Scientist

- 60 year old, diabetic male
- Dies 13 hours after presentation in the emergency room
- Worked in Infectious Disease Research – no virulent strains

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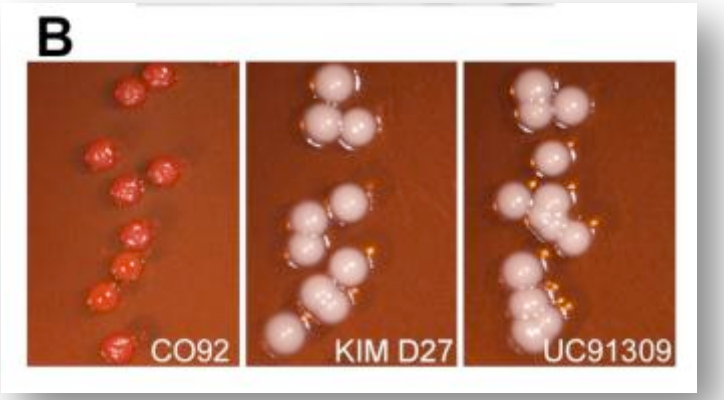
blood cultures:

- Gram-negative rods, Gram-positive cocci in chains, a yeast
- *Yersinia pestis*



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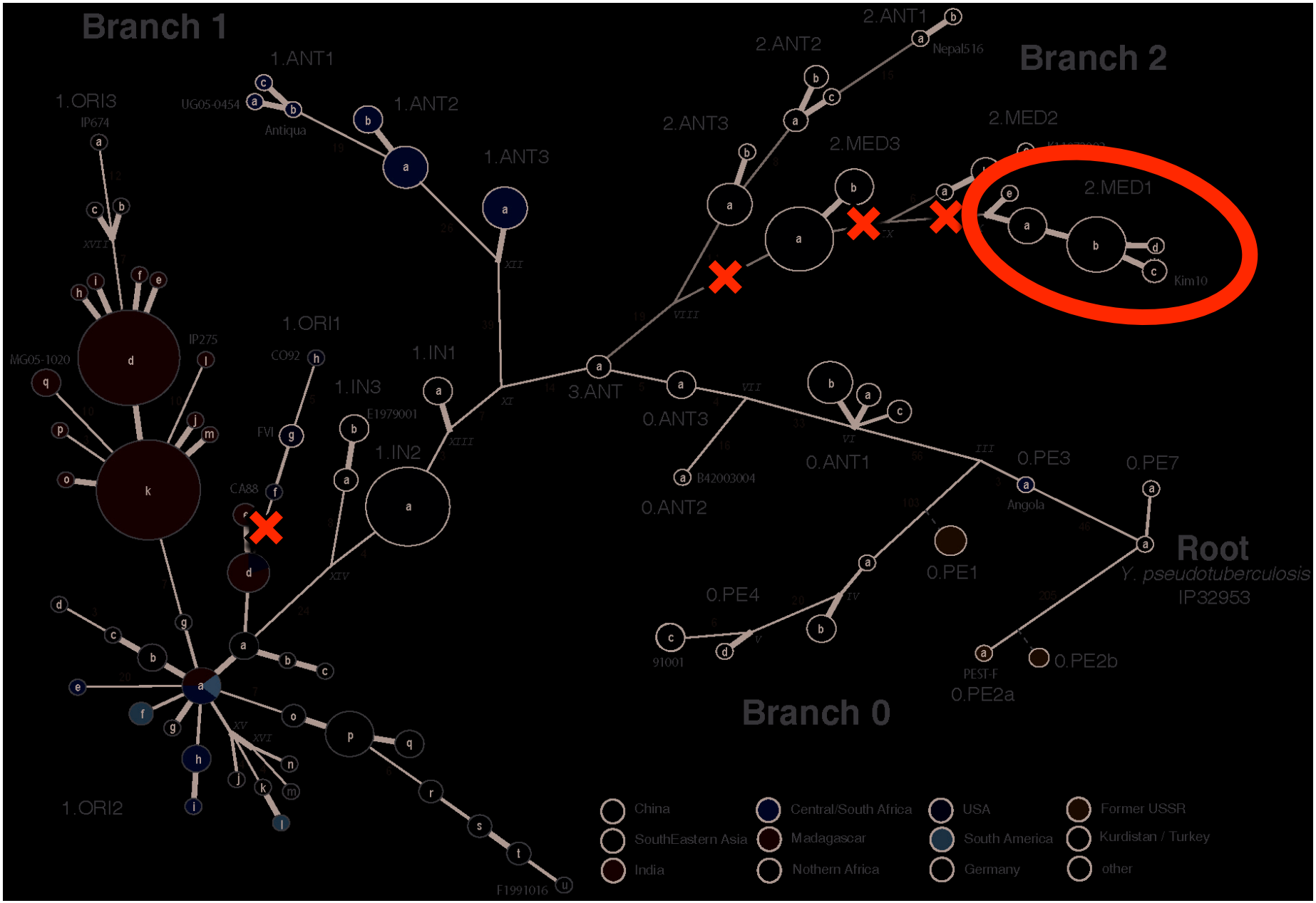
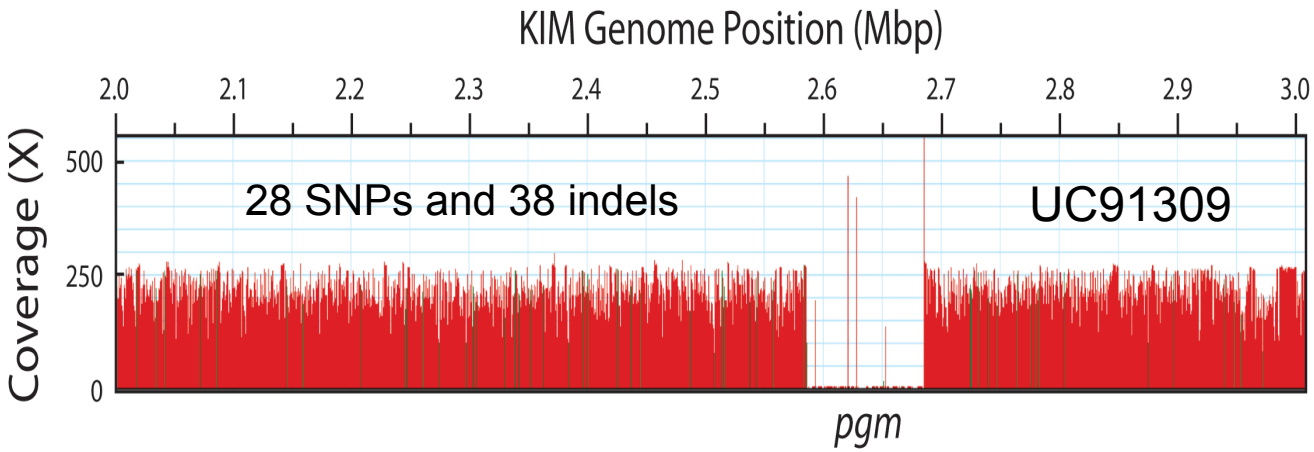


= analyzed canSNP

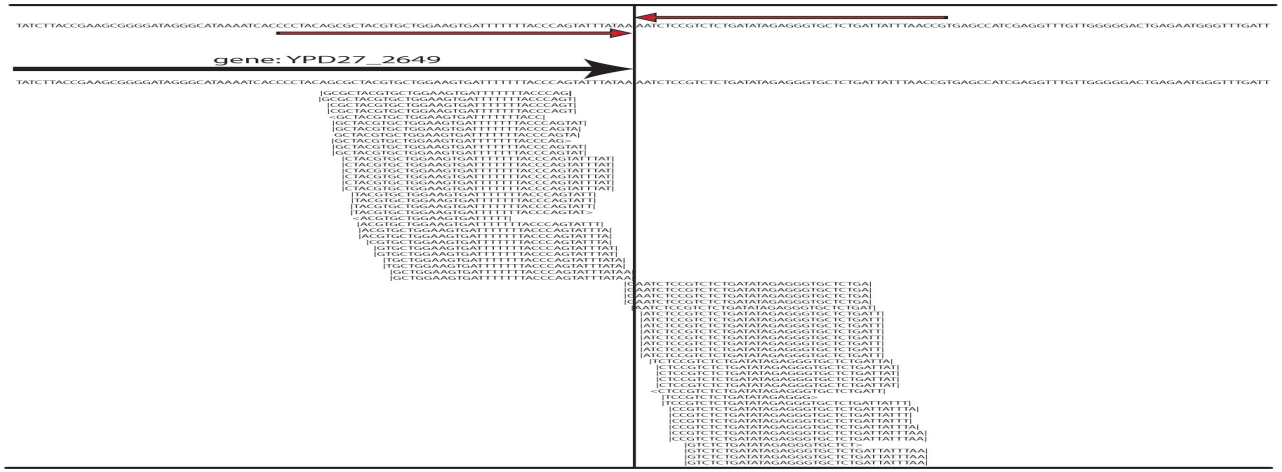
Morelli *et al*. Nature Genetics *2010*

Whole Genome Sequencing

A.
Attenuation
Defect
Unaltered



B.
Genetically
Engineered
Strain



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Jim Schupp
Unpublished data

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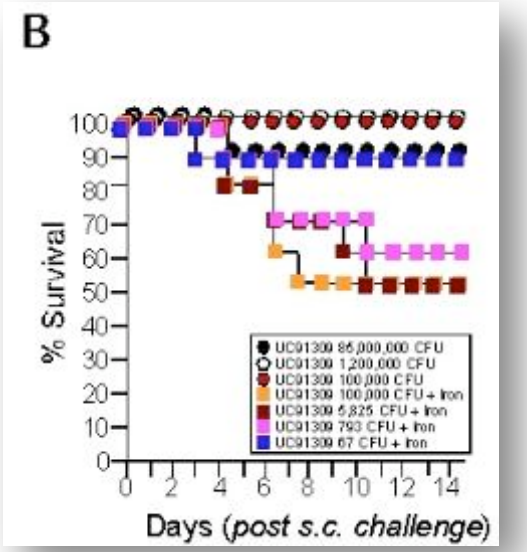
Animal Trials
UC91309



- Swiss Webster mice infected with *Y. pestis*
- 10^5 CFU – No deaths with KIM D27 or UC91309
- 10^2 CFU of CO92 - All mice died
- Supplement with iron – KIM/UC kills mice

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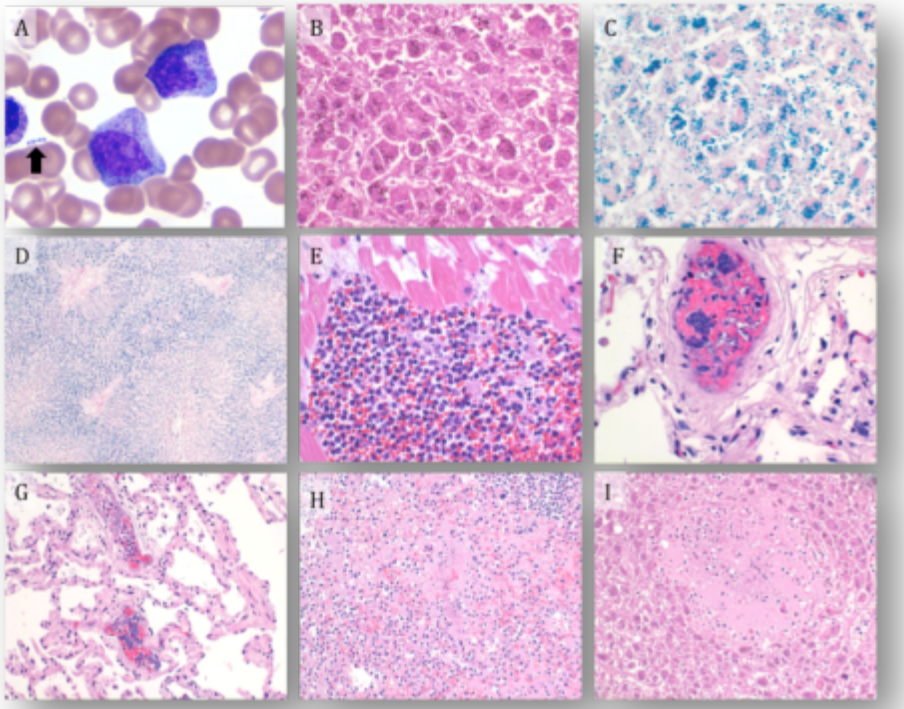
Summary

- Lab acquired infection
- *Y. pestis – KimD27*
- Genetically engineered
- Highly Attenuated
- Why was this lethal?

Frank et al.
unpublished data

Autopsy

- **Liver tissue - massive iron deposits.**
- **Human *HFE* Genotype: Homozygous C282Y**
- **Diagnosis of hereditary <u>hemochromatosis</u>**



Frank et al.

Unpublished data

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- Center for Microbial Genetics and Genomes
- Division of Pathogen Genomics

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Collaborators

- Mark Achtman – U of Cork
- Jacques Ravel – Un Maryland
- Ruifu Yang – Beijing
- Ken Gage, Jeanine Petersen, Michael Kosoy - CDC
- Suzanne Chanteau, Elisabeth Carniel – Institut Pasteur
- Pam Reynolds, Paul Ettestad – New Mexico Department of Health
- Olaf Schneewind, University of Chicago

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